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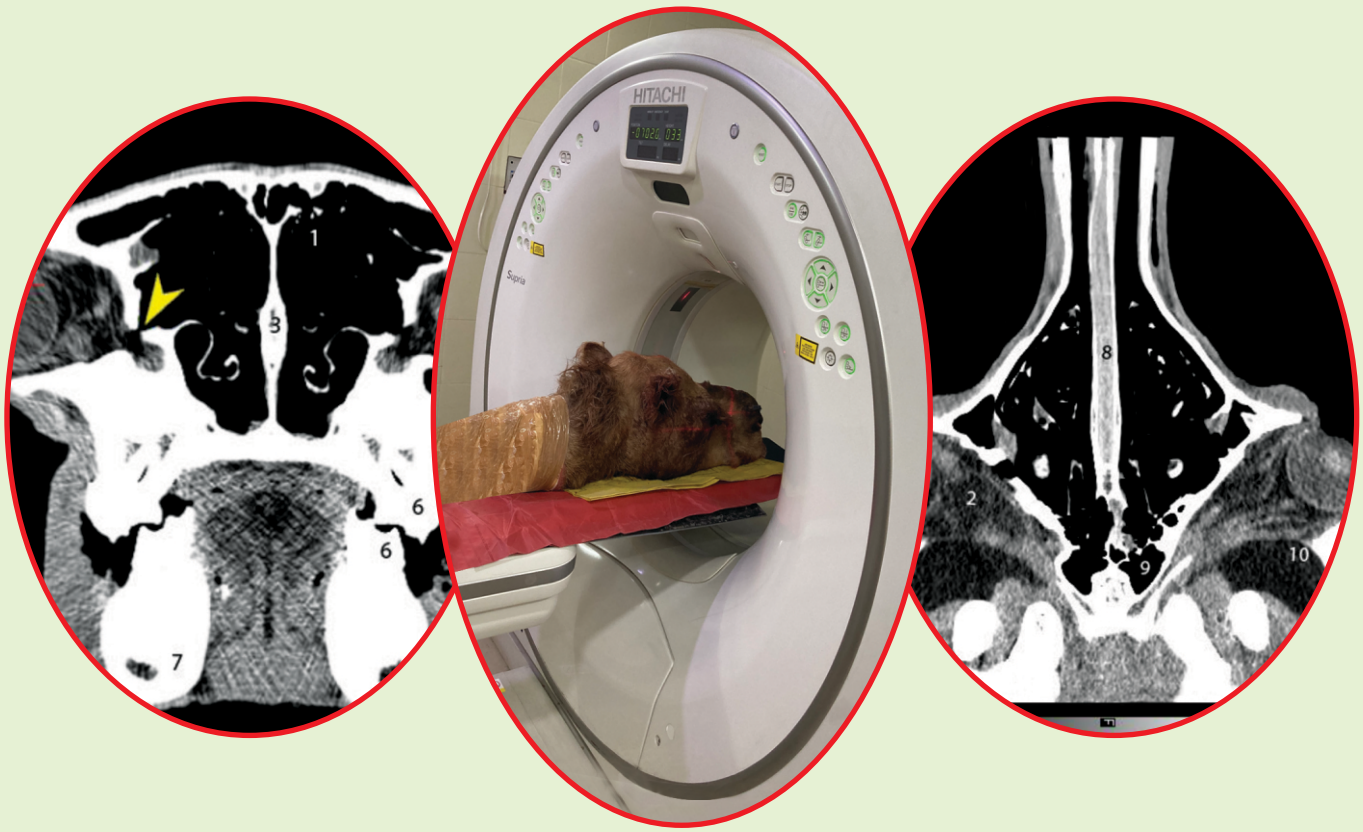

JOURNAL OF CAMEL PRACTICE AND RESEARCH

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

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Cover Photo: Positioning of cadaver head of adult camel in a 16 slice CT scan machine (Centre); Reformatted transaxial CT image of camel head demonstrating ocular structures (Left); Reformatted CT image in dorsal plane of camel head demonstrating ocular structures (Right)

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CAMELMILK PROJECT SUPPORT FROM EUROPEAN UNION AND FAO

The increase in demand for camel milk and products is not only translated into camel demography progress around the world, but also into multiplication of events as virtual scientific meeting (recently in Iraq, India, Pakistan, Kazakhstan, Morocco, Kenya, Sudan…), development projects and practical training in the dairy sector. Based on their experience in Saudi Arabia for processing camel milk into cheese, fermented milk and other milk beverages, Dr Bernard Faye (France) and Gaukhar Konuspayeva (Kazakhstan) have organized for the last 4-5 years several practical training on this topic, for example, in Mauritania and Chad with the support of FAO, at Laayoune in South-Morocco on the side-lines of the ISOCARD conference, at El-Oued in Algeria and Incirliova in Turkey in the frame of CAMELMILK project (supported by European Union), even in Canary Islands at Fuerteventura and at Montpellier in France (in CIRAD) where some farmers have switch their cattle to camel and aim to start milk and cheese production. The content of those training includes theoretical aspects and practical achievements where the trainees can make and taste their products. A guide for managing camel dairy farm was also edited, for the moment in Turkish and Arabic version. A Spanish and a French version will be available soon. Regular demands are coming from other countries such as Kazakhstan, Niger, or Oman. Camelmilk project scientists and cameleers are optimistic about great demand of camel milk products in supermarket in next decade.

The April 2022 JCPR has manuscripts based on research on dromedary and Bactrian, both. The new issue has an interesting review paper by Dr U. Wernery on *Rickettsiales* and *Coxiella burnetii* infections in camelids. Rickettsial bacteria were detected in healthy camels indicating the presence of asymptomatic carrier states. Q fever is a zoonotic disease caused by *Coxiella burnetii* but so far no disease has been attributed to Q fever in camelids, but many serological prevalences are found between 2 and 80%. Mahmoud Kandeel and his group of scientists carried out investigation on the role of insulin receptor in glycemic control in camels. Dr Sabry and El Hassan carried out research on production of an in-house rabbit anti-camel immunoglobulin G (IgG) conjugated with horseradish peroxidase (HRP) for use in immunoblots. Another important manuscript was on modern advances on the diagnosis of bovine viral diarrhoea virus in camelids was authored by Abdullah I.A. Al-Mubarak and associates. Screening of *Theileria annulata* in Saudi Arabia, computed tomographic imaging of eye, bacteria and fungi in the prepuce, female reproductive tract, Selected heavy metals and their risk assessment, low fat ice milk made from milk and defatted chia seeds flour, gross and morphometrical studies on different cervical vertebrae were important research on dromedary camels. Morphometric study on the gobi red bull and physical parameters of *Tülü* (Bactrian x Dromedary F1) calves were important studies on Bactrian camels.

I am sure that as Covid 19 pandemic is minimised, more interaction of camel scientists will take place through organising many camel conferences, workshops and trainings. International seminar on Promotion of camel milk value chain: Technology transfer and public-private partnership (ppp)- Camilk2022 shall take place from June 1-3, 2022 at Zarzis, Tunisia (https://camilk2022.com). I presume that many more announcements about camel conferences and other activities will come up later this year.

(Dr. Tarun Kumar Gahlot)
Editor

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Rickettsiales AND *Coxiella burnetii* INFECTIONS IN CAMELIDS: A REVIEW

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Overview

The members of the *Rickettsiales* Order are very small, non-motile, pleomorphic, obligate intracellular Gram-negative bacteria. They are coccobacilli or short rods, which are visible under light microscope best at 100 x oil magnification. *Rickettsiales* and *Coxiella* stain poorly with Gram but better with Giemsa and Romanowsky stains. Most of these bacteria do not grow on inert media. They require living cells for their replication and are normally cultured in tissue cultures (Munderloh *et al*, 2003), preferable tick cell cultures or in yolk sac of embryonated hen eggs (Passos, 2012).

The genus includes many species also associated with human disease, including those in the spotted fever and typhus group. The *Rickettsiae* that are pathogens to human beings are subdivided into three major groups based on clinical characteristics of the disease:

Spotted fever group with 8 species

Typhus group with 3 species

Scrub typhus group with 3 species

Rocky Mountain spotted fever caused by *Rickettsia rickettsii* for example is common in Mexico and North and South America and is transmitted by rodents, dog ticks like *Dermacentor* and *Amblyomma* species. In human beings the disease is characterised by fever, muscle pain, severe headache and occasionally by a myocarditis (Markey *et al*, 2013).

The classification of this group of bacteria is complex and complicated and not finalised, yet. For example, several species in the *Anaplasmataceae* family have been redesigned, as they previously included haemotrophic bacteria, which are now confirmed to be closely related to *Mycoplasma* as they also lack a cell wall.

The *Rickettsiales* Order comprises of two families of veterinary significance which are *Rickettsiaceae* and *Anaplasmataceae* (Markey *et al*, 2013). The family *Rickettsiaceae* possesses a cell wall, but members of the *Anaplamataceae* family lack a peptito glycan layer.

Significant re-classification of the Order has occurred several times over the years, which are mainly based on DNA sequencing in particular 16S and 23S-r RNA gene sequence comparisons. The classification is not yet complete.

The source of rickettsia taxonomy can be found in the latest (2004) edition of the Bergey's Manual of Systematic Bacteriology or under Schoch *et al* (2020), NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database Oxford 2020: baaa062. Pub Med: 32761142 PMC: PMC 7408187. This database gives an overview of *Rickettsiales* currently known. Most of them are either unclassified, uncultured or 'candidates' waiting for their classification; in total more 100 different species. However, the newest classification of *Rickettsiales* comprises the family *Rickettsiaceae* into two genera: Rickettsia and Orienta; both with no veterinary importance, but responsible for zoonotic diseases of human beings; while the family *Anaplasmataceae* have five genera: *Anaplasma, Ehrlichia, Neorickettsia, Aegyptianella* and *Wolbachia*.

Only few species of the *Anaplasmataceae* are pathogens of veterinary significance which are listed in Table 1.

As can be seen from this Table, none of them is mentioned to produce disease in camelids.

Coxiella burnetii, the cause of Q fever, is now closely related to *Legionella* species and *Franciscella tularensis* and is therefore dealt here in a separate section.

Natural Habitat and Pathogenesis

Members of the *Rickettsiales* are bacteria of arthopods which are replicating in the gut cells before spreading to other organs, such as salivary glands and ovaries. The requirement for an invertebrate vector, distinguishes these microorganisms from other bacterial species. This is unique. Infection

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Table 1. Rickettsial pathogens of veterinary significance according to Markey *et al* (2013).

Pathogen	Host	Vector	Country	Disease
Rickettsia rickettsii	Humans, dogs	Dermacentor species	Western hemisphere	Rocky Mountain spotted fever
Anaplasma marginale	Ruminants	Hard ticks	Tropics, sub-tropics	Gall sickness
A. ovis	Small ruminants	Hard ticks	Tropics, sub-tropics	Anaplasmosis
A. bovis	Cattle	Hyalomma	Africa, South America, Middle East, Asia	Bovine ehrlichiosis
A.platys	Canine	Ticks	America, Middle East, Mediterranean	Thrombocytopenia
A.phagocytophilum	Ruminants, horse, human beings	Ixodes	Worldwide	Tick-borne fever, equine and human granulocytic ehrlichiosis
Ehrlichia canis	Canine	Rhipicephalus	Tropics, subtropics	Canine monocytic ehrlichiosis
E. ewingii	Canine, human	Amblyomma	USA	Canine granulocytic ehrlichiosis
E. ovina	Ovine	Ticks	Africa, Asia, Middle East	Ovine ehrlichiosis
E. ondiri	Cattle	Ticks	East African highlands	Bovine petechial fever
Neorickettsia helminthoeca	Canine, bears	Salmonid fish ingestion	West Coast North America	Salmon poisoning disease
N. elokominica	Canine, bears, raccoons	Salmonid fish ingestion	North America	Salmon fever
N. risticii	Horse	Ingestion of aquatic insects	USA, Europe	Potomac horse fever
Aegyptianella pullorum	Birds	Argus species	Africa, Asia, Mediterranean	Aegyptianellosis

typically occurs as a result of a bite of an infected arthropod, mainly ticks. The pathogenesis varies widely with each species and a number of species persist in the host in a latent form. Identification of these microorganisms is not easy and is usually based on animal species infected, tick identification, clinical signs, demonstration of the bacteria in specimens, mainly blood, specific serological tests and PCRs. When the isolate has been obtained, sequence analysis of the genes should follow.

Rickettsiales in Camelids

Over the last decades several scientific papers have been published on tick-borne pathogens in camelids, either diagnosed during serological surveys or by molecular biological tools, especially PCRs using different primers. No publications were found describing culture methods in connection with this bacterial group. Some of the most important papers on rickettsial infections in camelids from different countries are found in Table 2. They also include *Rickettsiales* diagnosed in camel ticks. All investigations are so far snapshots and not long term studies. Additionally, with very few exceptions, most of these tests described in these publications have not been evaluated for use in camelids and all positive results were more or less from healthy camelids, showing no signs of illness with very few exceptions. Evaluation of serological tests is a prerequisite for a proper diagnosis as was recently shown by Soellner *et al* (2018), who evaluated many serological tests for the diagnosis of brucellosis in experimentally infected dromedaries. Parvizi *et al* (2020) evaluated a competitive ELISA for screening anaplasmosis, better *Anaplasma* infections, in camel populations in Egypt. Additionally, interpretation of results, where only staining methods were performed for the diagnosis of rickettsial infections, should be dealt with caution (Schuster *et al*, 2021), as it is often very difficult, if not impossible to diagnose intraplasmatic *Rickettsiae* correctly in blood smears. Some authors also exaggerate the effect of rickettsial infections in camels as causing significant losses in this species (Parvizi *et al*, 2020) or naming them "camel haemopathogens" (Kidambasi *et al*, 2020). So far only minor disease if any has been described in camelids and therefore one should use the word rickettsial infection instead of Rickettsiosis. It is also worthwhile mentioning, that *Anaplasma* species identified by PCR are named "*Candidatus Anaplasma*" (Lbacha *et al*, 2017), but other researchers are more confident that they have detected a new species that they named *Anaplasma camelii* without giving proper details. Some of these *Anaplasma candidatus* resemble

Table 2. Details of Rickettsiales species found in camelids and their ticks.

Rickettsiales Infection species	Authors	Test Kits	Results	Disease	Country
A. marginale, bovis, centrale	Wernery *et al* (2007)	cELISA VMRD, France	Blood 0.5% (5/1119)	None	UAE
A. marginale	Wernery *et al* (2014b)	PCR	Blood 0.0% (0/55)	None	UAE
A. marginale	Parvizi *et al* (2020)	cELISA PCR	Blood 1.6% (7/437) 1.6%	None	Egypt
Ca. A. camelii	Lbacha *et al* (2017)	PCR Gene: groEL	Blood 39.6% (42/106)	None	Morocco
Ca. E. regneryi *Ca. A. camelii* *C. burnetii* *Ca. E. regneryi* *Ca. A. camelii* *C. burnetii* *E. chaffeensis* *R. africae* *R. aeschlimannii*	Getange *et al* (2021)	PCR (DNA detection) PCR	Blood/ Ticks 80.1% (240/296) Camel Ticks *Hyalomma* *Amblyomma* *Rhipicephalus*	None	Kenya
A. platys	Li *et al* (2015)	PCR	Ticks (*Rhipicephalus sanguineus*) 7.2 % (20/279)	None	China
Ca. A. camelii	Kidambasi *et al* (2020)	PCR	Blood/ Ticks 68.67% (172/249) Camel Ticks *Hippobosca camelina*	None	Kenya
A. platys	Rassouli *et al* (2020)	PCR	Blood 3.3 % (2/60)	None	Iran
Ca. A. camelii	Sharifiyazdi *et al* (2017)	PCR	Blood 6.0 % (6/100)	None	Iran
E. ruminantium *E. canis* *Ca. E. regneryi*	Younan *et al* (2021)	PCR	Blood 2 camels	Heartwater-like disease	Kenya
A. phagocytophilum	Bahrami *et al* (2018)	PCR	Blood 34.2% (71/207)	None (subclinical?)	Iran
R. aeschlimannii *R. africae*	Kleinerman *et al* (2013)	PCR	Ticks 4.9% (3/148)	None	Israel
A. platys *A. canis*	Bastos *et al* (2015)	PCR	Blood 30.0% (30/100)	None	Saudi Arabia
A. phagocytophilum *A. marginale* *A. ovis* *Ca. A. camelii* *A. platys*	Azmat *et al* (2018)	PCR	Blood 13.3% (45/100)	Decreased white blood cell count	Pakistan
R. aeschlimannii *R. monacensis* *R. helvetica* *R. africae*	Selmi *et al* (2019)	Omp PCR	Blood 2.7% (8/293) *Hyalomma impeltatum* (10.4%) *H. dromedarii* (8.0%)	Not mentioned	Tunesia
A. platys	Belkahia *et al* (2015)	qPCR	Blood 17.7 % (40/226)	None	Tunesia

Abbreviations : A. = Anaplasma Ca. = Candidatus E. = Ehrlichia R. = Rickettsia C. = Coxiella H. = Hyalomma

Anaplasma platys and may not be a new species until proven. The high prevalence of "*Ca. A. camelii*" in healthy camels especially in Kenya seems to be an indication that the bacterium is either subclinical or non-pathogenic (Getange *et al*, 2021).

Candidatus (C.) *Anaplasma (A.) camelii* can be transmitted not only by ticks but also by the camel specific ked *Hippobosca camelina* as described by Bargul *et al* (2021). The authors also reported that the haematophagus ked transmit these bacteria to mice and rabbits via blood feeding. Sudan *et al* (2014) successfully treated subclinical anaplasmosis (*A. marginale*) in one dromedary camel in India showing anaemia and depression with a combination of different drugs.

In 2016, Younan *et al* (2021) described a heart water-like disease in Kenya but also in other countries (Onyiche *et al,* 2020; Alshahrani *et al,* 2020) which had killed 2000 adult animals. Gross pathology

showed pulmonary oedema, hydrothorax and hydropericardium. In the blood from two sick dromedaries, Ehrlichia species were identified by PCR resembling *E. ruminantium*, *E. canis* and "Candidatus *E. regneryi*". It was not clear, if any of these species were involved in this outbreak. Infection rates of *E. ruminantium* between 5.2% and 12.4% were reported by Getange *et al* (2021) in Kenya. These camels did not show any signs of heart water.

Anaplasmataceae were also reported in South American camelids. A llama suffered from granulocytic anaplasmosis and a strain was sequenced resembling *A. phagocytophilum* (Wernery *et al*, 2014b; Barlough *et al*, 1997). It has also to be stressed that special *Rickettsiae* species are only found in special ticks.

To overcome the uncertainity of a *Rickettsiae* infection in camelids, experimental infections are necessary to investigate, if this bacteria group is pathogenic to camels. This is, however a challenge, as many different rickettsial species have been described to occur in dromedaries, the most important ones are summarised in Table 2 with details of authors, test kits used, results, country of origin and disease details. The findings in Table 2 include also details about camel tick species and *Rickettsiae* species found in them.

Q Fever

Coxiellosis is caused by a Gram negative coccobacillus *Coxiella burnetii*, which does not belong anymore to the *Rickettsiales*, as phylogenetic analyses showed, that *C. burnetii* is more closely related to *Legionella* and *Franciscella* than to *Rickettsia* genus. This microorganism resides and replicates in its host's monocytes and macrophages. Two forms exist, the large cell variant is a vegetative form found in infected cells and the small cell variant is the extracellular infectious form shed in urine, milk and faeces. It is also found in very high concentrations in placental tissue and amniotic fluid like *Brucella* organisms. The disease is enzootic in most areas, where cattle, sheep and goats are kept; it is also a zoonotic disease and is frequently diagnosed in human beings, who have occupational contact with risk animal species like goats. A detailed overview of Q fever in dromedaries is presented in the OIE book compiled by Wernery *et al* (2014a).

So far no disease has been attributed to Q fever in camelids, but many serological investigations have been performed, most of them with serological prevalences between 2 and 80%.

Although a high prevalences has been reported from some African countries, the serological incidences in human beings, for example in Chad, were very low. However, antibodies against *C. burnetii* have been found in high numbers of livestock handlers in association with small ruminants (Getange *et al*, 2021). Belkahia *et al* (2020) found a serological prevalence of 75.5% in Algerian camels, but all 184 blood samples were negative in the PCR. However, five ticks from these dromedaries were PCR *C. burnetii* positive. Wernery (2011) reported that 45 raw camel milk samples originating from serologically positive dairy camels, were all Q fever negative using PCR technology. It is also worthwhile mentioning, that in this camel dairy farm, no Q fever abortions were reported. This is contrary to Q fever infected small ruminants.

Further studies are needed to better understand the role of camels in the epidemiology of Q fever and especially if they are or their products possess a zoonotic risk.

Resumé

Rickettsial bacteria, especially *Anaplasma* species have been found in dromedary and Bactrian camels either in their blood or in ectoparasites attached to their skin by molecular biological techniques by many researchers. Only very few serological investigations were carried out and no bacterial culture methods. These microorganisms were detected in healthy camels indicating the presence of asymptomatic carrier states. This comes as no surprise as camels are regularly infected by many different tick species, even sometimes covered by them without showing any signs of illness.

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BIOINFORMATICS AND MOLECULAR MODELING OF THE CAMEL INSULIN RECEPTOR

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ABSTRACT

Camels are well known for their high blood glucose content and marked glucose tolerance. In order to understand the glycemic control in camels the role of insulin receptor was investigated. The camel insulin receptor sequence, structure model, and domain content were compared with those of humans and other vertebrates. The results indicated 100% identity rate in old world camels, 99.7% identity rate with new world camels and 96% with humans. There was a high identity rate among insulin receptors in domestic animals (82.3–100%). Despite the conserved features of the insulin 1 binding site (the main functional insulin molecules), the second insulin-binding site in camel insulin receptors showed interesting differences. Most of the sequence differences between human and camel insulin receptors were concentrated in the insertion domains (ID), particularly the ID-β loop. ID-α′~αCT′~ID-β, which is important for insulin receptor signal transduction, showed a greater positive electrostatic potential in camels. Such differences might be associated with the noticed hyperglycemia and insulin resistance in camels by affecting the movement of the α-CT helix which lies between the IDs and significantly affects the main insulin molecules, lowering the affinity at insulin site 1 and by affecting the transmission of the insulin signal to the intracellular domain.

Key words: Camel, dynamics, glucose, insulin receptor, insulin resistance, simulation

Camels have physiological, anatomical and behavioral adaptation mechanisms for survival in desert environment (Gebreyohanes and Assen, 2017). Badryyah *et al* (2005) found that plasma glucose level in camels was significantly higher than that of goats. Food deprivation decreases plasma glucose levels in both monogastric mammals and ruminants of similar size as the camel (Evans, 1971; Rule *et al,* 1985). However, serum glucose level of camels was maintained during fasting and was increased after feeding had commenced (Wensvoort *et al,* 2004). The glycemia increases from 20 to 80% without glucosuria after 10 days of water deprivation. The hypo-insulinemia would allow the camel to maintain a low basal metabolism by decreasing glucose use (Ouajd and Kamel, 2005). Guo *et al* (2021) found that fasting in Bactrian camels is accompanied by changes in the activation of insulin pathways in various camel tissues, normal insulin levels, and increased lipolysis and insulin resistance, which returns to normal after eating. Díaz-Medina *et al* (2017) found in suckling dromedaries, a natural state of hyperglycemia and an increase in insulin resistance with age, while decreasing their tolerance to glucose, insulin secretion and insulin sensitivity, with reduced signs of use of free fatty acids (NEFA) from fat mobilisation.

Glycemic control is managed by insulin in both ruminants and monogastric animals (Sasaki, 2002). Insulin and insulin-like growth factors (IGFs) constitute a fundamental family of hormone polypeptides that play definite physiological roles in mammals and other species. Insulin is the key regulator of the homeostasis of carbohydrates and influences the metabolism of lipids and proteins (Frampton *et al,* 2020; Saltiel and Kahn, 2001). Specific cell surface receptors are needed for the physiological functions of insulin, and subtle differences in the structure and function of the receptors may account for significant variations in the biological activity of hormones. Compared with human and bovine insulin, the camel insulin showed attracting thermostability

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that might modulates the camel insulin function (Ismail, 2021). Furthermore, camel milk has been utilised in the treatment of diabetics due to its camel insulin content (Ashraf *et al,* 2021; Zheng *et al,* 2021). In this context, camel insulin showed marked structural differences and stability due to several amino acids replacements (Kandeel *et al,* 2022).

Although many insulin-like peptides exist in invertebrates, yet only one insulin receptor has been identified (Ruvkun and Hobert, 1998). The structure of insulin receptors comprises covalently-linked homodimers composed of several structural domains (Rentería *et al,* 2008). The insulin receptor is related to the superfamily Tyrosine Kinase (RTK) subclass II (White, 2017), which is a class of transmembrane receptors where the extracellular domain bears the ligand-binding site and exerts its intracellular signaling through the activation of intracellular tyrosine kinase.

In camel, the levels of glucose vary widely and are higher than in other ruminants (Nazifi *et al,* 1998). The high blood glucose levels in camels were hypothesised to be related to a higher glucagon level in camels than in humans and other ruminants (Abdel-Fattah *et al,* 1999) or camels bearing a natural insulin resistance. Insulin resistance is characterised as a condition in which the sensitivity of the target cells to react to ordinary insulin levels is reduced (Boura-Halfon and Zick, 2009); hence, the ordinary insulin levels fail to trigger metabolic actions. Camel adaptations to dry weather include maintaining a high glucose level in the blood of up to 1300 mg/dL with a loss of water in the urine. In this way, the camel can hold plasma water in order to resist water deprivation (Yagil, 1985). Camel tissues have a poor insulin response and low insulin sensitivity (Kaske *et al,* 2001).

There is an information gap regarding the camel insulin receptor and its contribution to the observed higher blood glucose and apparent insulin resistance in camels. Present study is aimed to identify camel insulin receptor sequence, structure model, and biological aspects. This study will compare camel insulin receptors with human and vertebrate insulin receptors as well.

Materials and Methods

Retrieval of insulin receptor sequences

The sequences used in this study were retrieved from the available GenBank and protein database (https://www.ncbi.nlm.nih.gov/). The retrieved sequences and their database accession numbers were Human XP_011526290, *Camelus dromedarius* XP_031292936, *Camelus bactrianus* XP_010965094, *Camelus ferus* XP_032322128, Canine XP_005633279, Ovine XP_004008598, Mouse NP_034698, Swine XP_020939599, Bovine XP_027403423, Rabbit XP_008247399, and Equine XP_023500375. In addition to the old world camelids, new world camelid insulin receptors were also retrieved. The new world camelids insulin receptor sequence was retrieved for Alpaca (*Vicugna pacos*) XP_031545692.

Searching homologs and orthologues

The search for identical sequences was based on the non-redundant (nr) database at NCBI. The basic local alignment search tool was used to investigate potential similar hits (Madden, 2013).

Comparison of human and camel insulin receptor putative domains and motifs

The putative domain content was analysed using the domain prediction tool available at NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer *et al,* 2005). The requests for motif searches were submitted to the motif search tool at the Kyoto Encyclopedia of Genes and Genomes (https://www.genome.jp/tools/motif/).

Multiple sequence alignment

The sequences of domestic animals and human insulin receptors were aligned using the sequence alignment tool with the CLC genomic software (Qiagen software, Denmark).

Phylogenetic tree

The phylogenetic relations were constructed and viewed with the CLC genomics workbench by using the sequences of insulin receptors from domestic animals.

Proteomic and genomic tools

The bioinformatics resource portal tools (ExPASy) (Artimo *et al,* 2012; Gasteiger *et al,* 2003) and tools at EBI (Cook *et al,* 2018; Labarga *et al,* 2007) were used for the analysis of protein sequences.

Construction of molecular models

Requests for molecular models were submitted to the Swiss model server. The retrieved structure models were inspected for the correct outliers using the Molprobity server. The structure models were energy minimised and compared with human insulin receptors by using a variety of software, including

ICM Molsoft, Molegro virtual docker, and the CLC drug discovery workbench.

Results

Comparison of human and camel insulin receptors

The human and camel insulin receptor sequences were aligned, and the alignment statistics were retrieved. The sequence comparison of human and serum camel receptors is provided in Fig 1. Camel and human receptors shared high homology with the identity% at 96% and the number of different amino acids at 54 residues. Most of the residue differences were in the range of 750–800 (human sequence numbers).

Insulin receptor in camelids

The sequence of insulin receptors was compared in the old world camel species *C. dromedaries*, *C. ferus* and *C. bactrianus* (Fig 2A). There were no differences in the insulin receptors protein sequence in the old world camel species, and there was a 100% identity rate. In comparing the differences between insulin receptors in the old world and new world camels, a sequence alignment was performed, and the results of the alignment summary are provided in Fig 2B. There was a very high identity per cent of 99.7% between the *Vacugna pacos* insulin receptor and the old world camels. There were only four amino acid differences in the insulin receptor sequences.

Insulin receptor in humans, dromedary camels, and other animals

Multiple sequence alignments were generated for the insulin receptor sequences from humans, camelids, and several animal species (Fig 3). There were no great differences among the examined sequences. The identity% was from 82.3 to 100%. The lowest identity% was with the chicken insulin receptor (82.3%) followed by the mouse and rabbit receptors. In contrast, the identity% was not lower than 97% between human, camelid, bovine, ovine, swine, canine, feline, and equine insulin receptors.

Motif and domain content of camel insulin receptor

The domains and motif contents of human and camel insulin receptors were compared (Fig 4-7). All studies revealed the lack of any differences in the domain constituents or motif contents between the human and camel insulin receptors. The conserved domain contents in the human and camel insulin receptors included a tyrosine kinase catalytic domain,

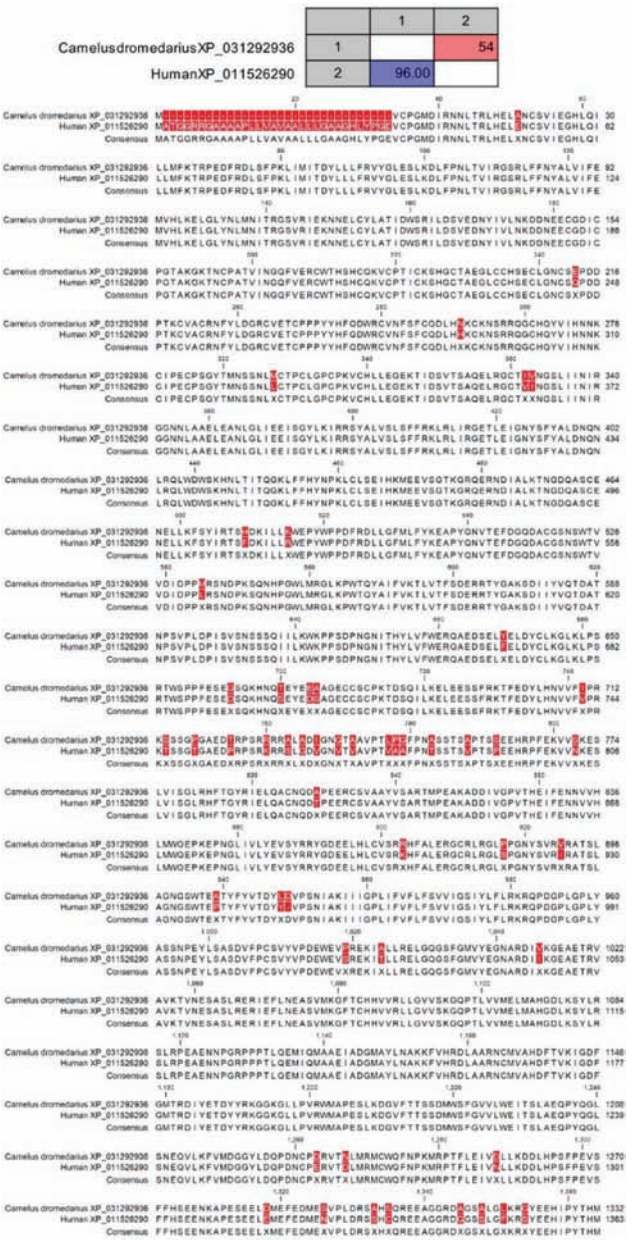


Fig 1. Pairwise alignment of human and camel insulin receptors. The upper panel shows the comparison statistics. The number of residue differences are highlighted in red and the identity% is highlighted in blue. The lower panel shows the sequence alignment. The different residues are in white and highlighted in pink.

receptor L domain, insulin receptor transmembrane segment, fibronectin type III domain, and furin-like cysteine-rich region.

Molecular models and insulin receptor structure

We constructed a molecular model of camel insulin based on the recently resolved human insulin receptor bond with insulin (PDB ID 6pxv). The

A

		1	2	3
CamelusdromedariusXP_031292936	1		0	0
CamelusferusXP_032322128	2	100.00		0
CamelusbactrianusXP_010965094	3	100.00	100.00	

B

		1	2	3	4
CamelusferusXP_032322128	1		4	0	0
VicugnapacosXP_031545692	2	99.70		4	4
CamelusdromedariusXP_031292936	3	100.00	99.70		0
CamelusbactrianusXP_010965094	4	100.00	99.70	100.00	

Fig 2. Summary of the multiple alignment statistics for the insulin receptor sequences. A) Comparison of old world camelids (*C. dromedaries, C. ferus*, and *C. bactrianus* insulin receptor). B) Comparison of the old world and new world camelid insulin receptor sequences. The upper right diagonal panel shows the number of residue differences. The lower left diagonal panel shows the identity.

		1	2	3	4	5	6	7	8	9	10	11	12	13
BovineXP_027403423	1		11	23	30	40	36	36	36	46	64	35	85	238
OvineXP_004008598	2	99.18		27	33	43	39	39	39	49	66	38	88	239
SwineXP_020939599	3	98.29	97.99		29	39	32	32	32	47	63	33	88	236
EquineXP_023500375	4	97.77	97.55	97.84		33	33	33	33	39	57	28	82	234
CatXP_023100560	5	97.03	96.80	97.10	97.55		45	45	45	51	63	29	91	237
CamelusdromedariusXP_031292936	6	97.32	97.10	97.62	97.55	96.65		0	0	54	69	38	93	238
CamelusferusXP_032322128	7	97.32	97.10	97.62	97.55	96.65	100.00		0	54	69	38	93	238
CamelusbactrianusXP_010965094	8	97.32	97.10	97.62	97.55	96.65	100.00	100.00		54	69	38	93	238
HumanXP_011526290	9	96.58	96.36	96.51	97.10	96.21	95.99	95.99	95.99		49	47	69	236
RabbitXP_008247399	10	95.24	95.09	95.32	95.76	95.32	94.87	94.87	94.87	96.36		61	85	247
CanineXP_005633279	11	97.40	97.17	97.55	97.92	97.84	97.17	97.17	97.17	96.51	95.46		87	239
MouseNP_034698	12	93.69	93.47	93.47	93.91	93.24	93.10	93.10	93.10	94.88	93.69	93.54		233
ChickenXP_001233399	13	82.33	82.26	82.48	82.63	82.41	82.33	82.33	82.33	82.48	81.66	82.26	82.56	

Fig 3. A summary of the multiple alignment statistics for the insulin receptors in humans and domestic animals. The upper right diagonal panel shows the number of residue differences. The lower left diagonal panel shows the identity. The cells are highlighted according to a color scale from red to blue, where red is the lowest and blue is the highest.

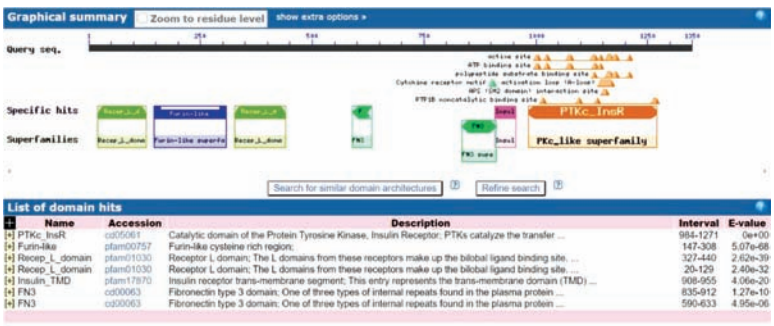


Fig 4. The human insulin receptor domain content. The results were generated using the NCBI domain search tool. The figure was generated using the NCBI domain server.

coverage rate was 99% of the input sequences with a sequence identity of 95.81%.

The domain composition of the insulin receptor included the leucine-rich domains L1 (52–163) and L2 (359-469), a furin-like cysteine-rich region (179-340), fibronectin type III domains (FNIII-1 629-663, FNIII-2 795-829, FNIII-3 863-936), an insulin receptor transmembrane segment (940-986), protein kinase domain or PK (1024-1287), RNA binding domain B2 (1052-1106), and kinase-like domain (1146-1277) (Fig 8).

The insulin S1 and S1`-binding site

The structure is composed of a dimer containing two insulin molecules per monomer. The first insulin in the first monomer was termed S1, and its corresponding insulin molecule on the second monomer was termed S1`. Similarly, the second insulins were termed S2 and S2` for the first and second monomers, respectively. The first insulin S1 molecule is placed between the L1 domain of one monomer, the FnIII-1` domain, and the α-CT` helix of the other monomer. There were no significant differences in the amino acid composition at the insulin-binding site of the insulin receptor between human and camel insulin receptors. In the L domain, there were three amino acid differences: E51A in the L1 domain and V363I and I364V. This indicates minor amino acid differences in the L domain. No other significant differences were found in the α-CT helix.

The insulin second S2-binding site

A potential second S2 insulin-binding site is proposed near the junction of the FnIII-1 and FnIII-2 fibronectin domains (McKern *et al,* 2006; Scapin *et al,* 2018). Insulin S2 interacts directly with the FNIII-1 domain and the loop connecting the FNIII-1 and FNIII-2 domains.

The differences between human and camel insulin receptors

Little differences were observed between the human and camel insulin receptor sequences. However, a large number of amino acid replacements were observed at the residue range of 746-803, which corresponded to the insertion domain beta (ID-β).

A common structure linking insulin S1 and S2 molecules

The ID-β loop was a common structure linking the binding of the insulin receptor with the two insulin S1 and S2 molecules. This loop was found to have most of the sequence differences

between human and camel insulin receptors.

In comparing the camel and human ID-β domain sequence, about 16 amino acid replacements were observed (Table 1.). In human ID-β, the different residues comprise four polar uncharged R-group, 10 nonpolar aliphatic R-group, and two charged residues. The camel ID-β lacks the charged residues, which are replaced by two nonpolar residues.

The surface electrostatic potential of human and camel ID-α~αCT~ID-β

Based on the observed differences in the composition of ID-α′~αCT′~ID-β between human and camel insulin receptors, the surface electrostatic maps were compared (Fig 9). The results indicated that the human ID-α′~αCT′~ID-β bears a higher negative electrostatic potential, which is replaced by a positive charge in camel ID-α′~αCT′~ID-β.

Discussion

In this study, a trial was adopted to understand the potential contributing factors for the resistance of camels to high glucose contents by analysing the potential differences between camel, human, and other vertebrate insulin receptors.

The L1 subdomain and the α-CT helix are essential for insulin binding (De Meyts and Whittaker, 2002). More recently, the α-CT helix and CR domain are important in binding insulin-galgrine (González-Beltrán and Gómez-Alegría, 2021). As there were no amino acid differences in the L1 domain, the FnIII-1 domain, and the α-CT helix between human and camel insulin receptors, the binding mode of the insulin-1 molecule with the insulin receptor appeared to be conserved between humans and camels. However, recent studies have revealed the importance of structural rearrangements in the formation of insulin binding sites (Gutmann *et al*, 2020).

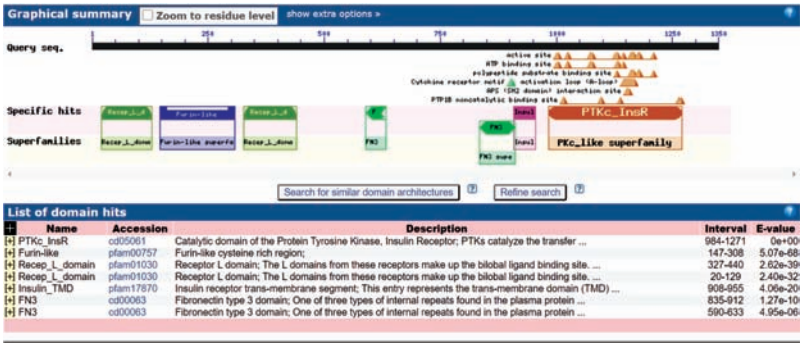
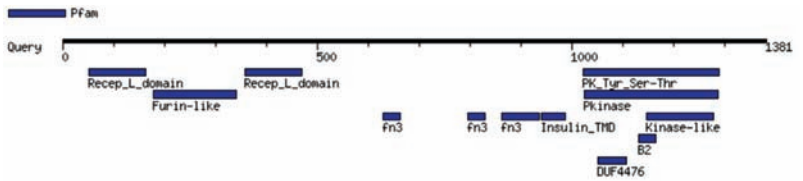


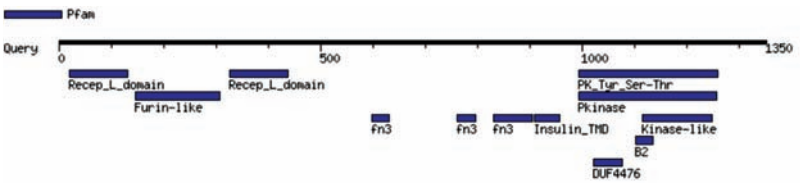
Fig 5. The camel insulin receptor domain content. The results were generated using the NCBI domain search tool. The figure was generated using the NCBI domain server.



Pfam (9 motifs)

Pfam	Position(Independent E-value)		Description
PK_Tyr_Ser-Thr	1023..1289(9.8e-95)	Detail	PF07714, Protein tyrosine and serine/threonine kinase
Recep_L_domain	52..163(2.9e-25) 359..469(6e-29)	Detail	PF01030, Receptor L domain
Pkinase	1024..1287(6.2e-41)	Detail	PF00069, Protein kinase domain
Furin-like	179..340(3.6e-36)	Detail	PF00757, Furin-like cysteine rich region
Insulin_TMD	940..986(2.2e-21)	Detail	PF17870, Insulin receptor trans-membrane segment
fn3	629..663(0.00044) 795..829(0.56) 863..936(3e-05)	Detail	PF00041, Fibronectin type III domain
Kinase-like	1146..1277(0.0045)	Detail	PF14531, Kinase-like
B2	1132..1165(0.33)	Detail	PF11473, RNA binding protein B2
DUF4476	1052..1106(0.32)	Detail	PF14771, Domain of unknown function (DUF4476)

Fig 6. The human insulin receptor motifs content. The results were generated using the KEGG motifs search tool. The figure was generated using the KEGG motif search server.



Pfam (9 motifs)

Pfam	Position(Independent E-value)		Description
PK_Tyr_Ser-Thr	992..1257(1.4e-94)	Detail	PF07714, Protein tyrosine and serine/threonine kinase
Recep_L_domain	20..131(2.8e-25) 327..437(1.7e-28)	Detail	PF01030, Receptor L domain
Pkinase	994..1255(4.1e-40)	Detail	PF00069, Protein kinase domain
Furin-like	147..308(2.5e-36)	Detail	PF00757, Furin-like cysteine rich region
Insulin_TMD	908..955(1.7e-20)	Detail	PF17870, Insulin receptor trans-membrane segment
fn3	597..631(0.00045) 760..796(0.75) 831..904(2.1e-05)	Detail	PF00041, Fibronectin type III domain
Kinase-like	1115..1246(0.0032)	Detail	PF14531, Kinase-like
B2	1101..1134(0.32)	Detail	PF11473, RNA binding protein B2
DUF4476	1021..1075(0.31)	Detail	PF14771, Domain of unknown function (DUF4476)

Fig 7. The camel insulin receptor motifs content. The results were generated using the KEGG motifs search tool. The figure was generated using the KEGG motif search server.

Table 1. The different residues between human and camel insulin receptor at the ID-β domain. Position number is according to the human insulin receptor sequence.

Position	Residue in human	Nature of residue in human	Residue in camel	Nature of residue in camel
746	Threonine	Polar uncharged R-group	Serine	Polar uncharged R-group
750	Threonine	Polar uncharged R-group	Proline	Nonpolar, aliphatic R-group
755	Proline	Nonpolar, aliphatic R-group	Threonine	Polar uncharged R-group
760	Lysine	Basic charged (positive charge)	Proline	Nonpolar, aliphatic R-group
763	Serine	Polar uncharged R-group	Alanine	Nonpolar, aliphatic R-group
765	Glycine	Nonpolar, aliphatic R-group	Alanine	Nonpolar, aliphatic R-group
767	Valine	Nonpolar, aliphatic R-group	Isoleucine	Nonpolar, aliphatic R-group
770	Valine	Nonpolar, aliphatic R-group	Glycine	Nonpolar, aliphatic R-group
772	Valine	Nonpolar, aliphatic R-group	Alanine	Nonpolar, aliphatic R-group
777	Valine	Nonpolar, aliphatic R-group	Leucine	Nonpolar, aliphatic R-group
778	Alanine	Nonpolar, aliphatic R-group	Proline	Nonpolar, aliphatic R-group
779	Alanine	Nonpolar, aliphatic R-group	Glycine	Nonpolar, aliphatic R-group
783	Threonine	Polar uncharged R-group	Alanine	Nonpolar, aliphatic R-group
788	Valine	Nonpolar, aliphatic R-group	Alanine	Nonpolar, aliphatic R-group
792	Proline	Nonpolar, aliphatic R-group	Serine	Polar uncharged R-group
803	Asparagine	Acidic charged (negative charge)	Serine	Polar uncharged R-group

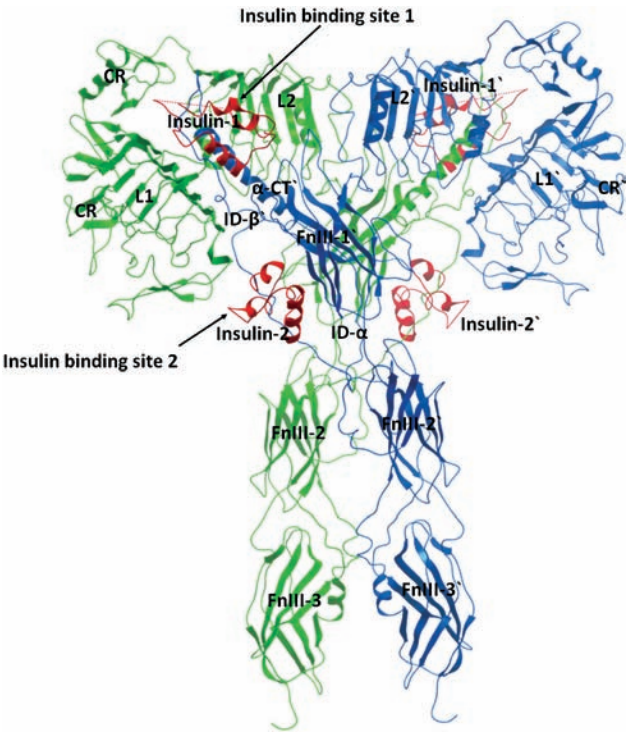


Fig 8. A structure model of the camel insulin receptor ectodomain showing different domains.

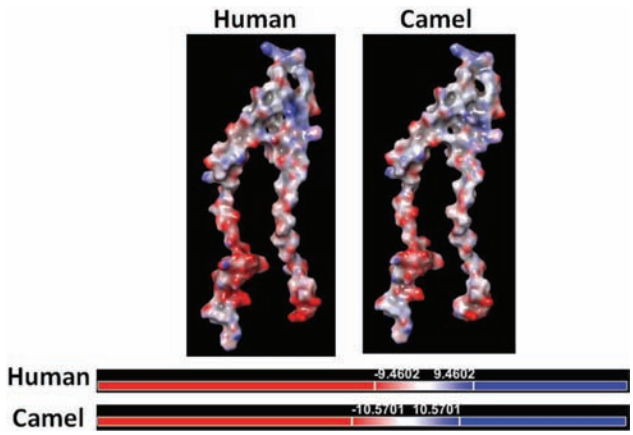


Fig 9. The surface electrostatic potential of ID-α′~αCT′~ID-β in humans and camels. The negative charge is displayed in red. The positive charge is displayed in blue. The neutral residues are in white.

The molecular dynamics of full-length insulin receptors revealed important structural changes of insulin receptors. Interestingly, the ID-α′~αCT′~ID-β′ domains form a long loop around the binding sites of insulin. Structure rearrangements involving the ID-α′~αCT′~ID-β′ are essential to allow the formation and access of insulin to its first binding site (Yang *et al,* 2020).

The concept of the binding of four insulin molecules to insulin receptors was proven on a structural basis (Gutmann *et al,* 2020). However, the exact interactions of these four insulin molecules as well as the insulin receptor structural changes and signal transduction are still not understood. Several assumptions were made to implement the contribution of the four insulin molecules to the insulin receptor function. In association with these speculations, the binding of insulin at site 2 is weaker than at site 1; therefore, insulin dissociates from

site 2 upon formation of the strong binding site 1. As site 2 is very close to site 1 and comprises the binding of insulin 2 with the L1′ domain and the αCT helix, the second site might be essential for favorable conformational changes in site 1 to achieve the proper binding of insulin 1 by enhancing favorable structural changes (Gutmann *et al,* 2020; Yang *et al,* 2020).

There was also an important difference between the conformation of the bound insulin at sites 1 and 2. The insulin at site 1 was in open or receptor-bound conformation, while insulin at site 2 was in closed form, similar to insulin in solution or not bound to the insulin receptor (Gutmann *et al,* 2020). The additional interaction of insulin 2 with ID loops was observed. There was an asymmetric binding of insulin 2 with IDs loops and their αCT/αCT` helices, which are critical for high-affinity binding. The conformational changes from the L1~CR~L2~Fnш-1 and ID-α~αCT~ID-β domains are transmitted through the transmembrane domain and induce structural and functional changes in the intracellular domain of the insulin receptor.

Previous studies on the hagfish (*Myxine glutinosa*) insulin receptor indicated a conserved insulin site 1 residue composition. However, insulin binding showed slow kinetics and a low binding affinity (De Meyts, 2004). This was attributed to differences in two site 2 residues. Similarly, the camel insulin receptor bears several differences in the site 2 composition that might contribute to the observed low insulin sensitivity in camels.

In ruminants, the mechanism of action of insulin on the glucose metabolism is similar to monogastric animals. Glucose transport controls the rate of glucose utilisation. In skeletal muscles and adipose tissues, there are three glucose transporters: Glucose transporter 1, 3, and 4 (GLUT1, GLUT3, and GLUT4) (Sasaki 2002). The insulin-sensitive glucose transporter GLUT4 (as with monogastric animals) is believed to be the key protein in the regulation of the glucose uptake and metabolism in ruminants. By inducing the translocation of GLUT 4 from an intracellular membrane pool to the plasma membrane in adipocytes and muscles, insulin controls the glucose transport.

In addition, insulin-induced GLUT4 translocation is triggered via the typical intracellular signaling pathway of the signaling pathway of insulin. The process of the translocation of GLUT4 mRNA and protein and insulin-induced GLUT4 on adipocytes and muscles in ruminants is slower than in rodents and human subjects. Despite a normal status, the resistance of insulin to the stimulatory action of the glucose metabolism in ruminants in comparison with monogastric animals could be attributed to the lower GLUT4 content and lower insulin signal transduction ability, which lead to decreased glucose transport operations (Sasaki, 2002). The translocation of signal and the observed changes in GLUT4 activity might be attributed to the observed camel receptor structure differences.

Combined together, the camel insulin binding receptor bears several indicators of low insulin activity comprising conserved insulin 1 site composition, the presence of several mutations in the insulin site 2 composition, a different electrostatic potential of ID-α′~αCT′~ID-β, and a low homology of ID loops—in particular ID-β. The changes in insulin site 2 and the ID loops might extend their effects to the αCT helix and decrease the insulin binding affinity. The ID-α~αCT~ID-β in the camel structure appears to be domain with the most influence. The different ID loop compositions might affect the signal transduction to the signaling bridge and modulate the intracellular insulin signaling.

In conclusion, there was a high identity rate among insulin receptors in domestic animals (82.3–100%), while human and camel insulin receptors shared 96% identity. Despite the conserved features of the insulin 1 binding site (the main functional insulin molecules), the second insulin-binding site in camel insulin receptors showed interesting differences. Most of the sequence differences between human and camel insulin receptors were concentrated in the insertion domains, particularly the ID-β loop. Such differences might be associated with the noticed hyperglycemia and insulin resistance in camels by 1) affecting the movement of the α-CT helix, which lies between the IDs and significantly affects the main insulin molecules, 2) lowering the affinity at insulin site 1, and 3) affecting the transmission of the insulin signal to the intracellular domain. These findings contribute to our knowledge regarding glycemic control and the function of the insulin receptors in camels.

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Human and animal rights

No animals / humans were used for the studies that are basis of this research.

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Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

MK designed and performed this study. MG, MB, SA and SI analysed data, and MK wrote the manuscript. MG, MB, SA and SI revised the manuscript. All authors approved the final version of the manuscript.

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PRODUCTION OF AN IN-HOUSE RABBIT ANTI-CAMEL IMMUNOGLOBULIN G (IgG) CONJUGATED WITH HORSERADISH PEROXIDASE (HRP) FOR USE IN IMMUNOBLOTS

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ABSTRACT

The present study was aimed to develop anti-camel immunoglobulin G (IgG) conjugate. Camel serum was obtained from healthy camels and the IgG was extracted from this serum using the commercially available IgG purification kits. The electrophoretic profile of camel IgG showed four bands of molecular weight 66, 50, 44 and 33 kDa. Two rabbits were immunised with the purified camel IgG for production of rabbit anti-camel IgG. IgG fraction from serum collected from the immunised rabbits at the end of the immunisation protocol was purified using the commercially available IgG purification kits. The purified IgG tested for reactivity using ELISA against camel serum and then coupled to horseradish peroxidase. The produced conjugate then tested for reactivity using western immunoblotting against serum from camels infected with *Haemonchus longistipes* and normal camel serum. The conjugate was able to react with camel serum and was able to detect three components of *H. longistipes* of molecular weight 126, 76 and 18 kDa.

Key words: Conjugation, dromedary camels, *H. longistipes,* HRP, IgG, immunoblots

Immunoassays are the most popular techniques for diagnosis of infectious diseases and are dependent on the availability of anti-species conjugates, particularly those used for antibody detection (Aregawi and Feyissa, 2016). Different types of labeling systems are available depending on the proposed assay. Fluorochromes, such as fluorescin isothiocyanate (FITC) and tetramethyl rhodamine isothiocyanate (TRITC) are generally suitable for immunohistochemistry and flow cytometry (Johnstone and Thorpe, 1982; Poojan and Kumar, 2011; Avens and Bowman, 2010). Radioisotopes such as iodine (125I) are used in radioimmunoassay (Liddell and Cryer, 1991; Zahi *et al,* 2010), while enzymes are used for increasingly popular enzyme-immunoassays (Ivey *et al,* 2011).

Enzymes have achieved a high degree of popularity in immunoassays mainly because of their high specificity for the substrate, which can be, converted to coloured products that can be either soluble or insoluble. Enzymes also have the advantage over other labels in that they are readily available, inexpensive and have high specific activity (Tijssen, 1985). The most commonly used enzymes include horseradish peroxidase (HRP), alkaline phosphatase (AP) and β-galactosidase (β-G) (Greenfield, 2014). Overall, HRP conjugates are considered to be superior to AP and ß-G conjugates due to the higher specific enzyme activity and immunological reactivity of HRP conjugates (Porstmann *et al,* 1985). The reaction products of HRP are easily detectable, while that of AP and β-G are less strongly coloured (Liddell and Cryer, 1991).

There are a number of methods used to couple HRP to immunoglobulin, however, the periodate method (Wilson and Nakane, 1978) is the most commonly used. This method is popular because it couples the antibody to HRP through the carbohydrate portion of each molecule and since this is not usually involved in the active site of the antibody and the enzyme, the method is less likely to reduce the immuno-reactivity. Immunoassays such as western immunoblotting depends mainly on the availability of anti-species conjugates. Commercial anti-species conjugates can be purchased from the market, however, anti-camel conjugates are too

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expensive in these markets. The present study was aimed to develop anti-camel immunoglobulin G (IgG) conjugate.

Materials and Methods

Parasites

Adult *Haemonchus longistipes* worms were obtained from the abomasa of naturally infected slaughtered camels at Al-Ahsa Central Abattoir, KSA following the method of Smith and Smith (1996). The examined abomasum contained only *H. longistipes* parasites identified by their barber pole appearance and long spicules (Soulsby, 1986).

Serum samples

Blood samples were collected from the jugular vein of *Haemonchus longistipes* infected and uninfected camels and from the ear vein of the immunised rabbits into glass bottles without anticoagulant for serum preparation.

Production of polyclonal antibodies to camel immunoglobulin

Two rabbits were immunised with the purified camel IgG using an immunisation regime adapted from Greenfield (2014). Each rabbit was injected intramuscularly with 100µg antigen emulsified in FCA on day 0 and on day 14. Two booster doses of 50µg antigen in FIA were administered to each rabbit subcutaneously on day 49 and 77 and a final boost of 25 µg of antigen in FIA on day 196. Serum was collected from these rabbits on days -1, 24, 56, 84, 203 and 210.

Monitoring antibody production

An antibody-ELISA was used to measure the amount of antibody in the sera collected from the immunised rabbits on days 24, 56, 84 and 203, post primary injection of the immunogen and the titre of the final serum collected on day 210. Each serum was tested against the purified camel IgG at a dilution of 1/100 as antigen. Sera were evaluated over a 2-fold dilution range from 1/250 – 1/8000 for the sera collected 24-203 days and over a 2-fold dilution range from 1/500-1/1024000 for the final serum collected at day 210. In each case, pre-immunisation serum at a similar dilution range was included as a negative control. All serum samples were tested in duplicates and the assay performed using a peroxidase labelled anti-rabbit IgG conjugate. Serum samples showing an absorbance value greater than two standard deviations (2xSDEV) above the mean of the negative control were considered positive. The antibody titre of the final serum was taken as the last dilution that continued to show an absorbance of more than 2xSDEV above the mean of the negative control.

Purification of IgG antibodies from serum

The IgG from camel serum and serum raised in rabbits against camel IgG was purified using the commercially available ImmunoPureR IgG kit (Pierce Chemical Company, USA). The method utilising 1ml columns of immobilised protein A covalently coupled to 6%, crosslinked, beaded agarose (Protein A affinityPakTM columns). Fractions eluted from these columns were dialysed against PBS. The concentration of the purified camel IgG is measured using the commercially available BCA kit. Antibody reactivity of dialysed rabbits IgG fractions was tested against the purified camel IgG by antibody-ELISA. The purified camel IgG was used at 1/100 dilution. Rabbit IgG preparations were tested at an eight 2-fold dilutions from 1/100 to 1/12800. Normal rabbit serum at similar dilution series was also included in the test as a negative control. IgG preparations dilution showing an absorbance value greater then 2xSDEV above the mean of the similar dilution of the negative control was considered positive.

Detection of purified camel IgG by SDS gel electrophoresis (SDS-PAGE)

Purified IgG samples were electrophoresed on 7-20% gradient polyacrylamide gels as described by Laemmli (1970). Protein banding pattern on the gels was visualised by Coomassie Blue stain and their molecular weight were determined and compared to pre-stained protein ladder of molecular weights ranged from 10-200 kDa (Cell Signaling Danvers, MA, USA).

Labeling IgG antibodies with horseradish peroxidase

Before labeling, the selected IgG fractions were dialysed with slow stirring overnight against several changes of distilled water at 4°C and freeze-dried overnight using a freeze-dryer (LABCONCO, USA). These lyophilised IgG fractions were then conjugated to HRP (EC 1.11.1.7; CAS RN 9003-99-0; Sigma-Aldrich, Inc. Missouri, USA) using the modified periodate method (Wilson and Nakane, 1978). The resulting IgG-HRP conjugate was dialysed overnight against PBS at 4°C. Prior to storage an equal volume of glycerol was added to the conjugate which was then stored at -20°C until needed.

Reactivity of labeled IgG antibodies in immunoblotting

H. longistipes crude soluble extract was stored frozen in SDS-sample buffer and heated

immediately before use to 100°C for 3 minutes to re-dissolve any crystallised SDS. Cooled samples were then electrophoresed on 7-20% gradient polyacrylamide gels as described by Laemmli (1970). After electrophoresis, Western blots were prepared by electro-blotting separated antigens in unstained gels onto nitrocellulose paper according to the method described by Towbin *et al* (1979) and Burnette (1981) using a Trans-Blot SD semi-dry transfer cell (Bio-Rad, USA). Antigens were detected by western immunoblotting against serum from camels infected with *H. longistipes* and normal camel serum as a negative control. The unbound sites on the nitrocellulose membranes were blocked overnight with 5% dried milk in blocking buffer (50mM Tris, 150mM sodium chloride, 1mM EDTA, 0.05% NP40, 0.25% gelatin, 0.02% thimerosal, pH 7.4) prior to each incubation. The blocked membrane was rinsed with PBS, cut into strips and incubated overnight with the above sera diluted 1/50 in blocking buffer. The incubation was carried out at room temperature. The unbound antibodies were then removed by washing the membranes with 7 changes of PBS over 2 hours, before the produced peroxidase-labelled, rabbit anti-camel IgG diluted to 1/200 in blocking buffer was added and incubated for 2 hrs. After washing the membranes in PBS as above to remove excess conjugates, the labeled antigen/antibody complexes were visualised by incubation in substrate solution (4-chloro-1-α-naphthol 0.5 mg/ml in 20 mM Tris, 500 mM sodium chloride, pH 7.5, Sigma Ltd., UK) with hydrogen peroxide (0.06%) as the substrate. Colour development was allowed to occur for 30 minutes and the reaction was stopped by washing in distilled water and permanent records made by photography.

Results

SDS gel electrophoresis (SDS-PAGE) profile of purified camel IgG

The electrophoretic profile of camel IgG showed four bands of molecular weight 66, 50, 44 and 33 kDa (Fig 1).

Monitoring antibody response in immunised rabbits

Serum collected from the immunised rabbits on days 24, 56, 84 and 203 following primary injection of the antigen showed a progressive increase in absorbance values when tested by ELISA against purified camel IgG (Fig 1). At a serum dilution of 1/250 approximately 4-fold increase in the absorbance value from day 24 to day 203-post primary injection was observed. The anti-serum showed an absorbance value of 0.624 at day 24 which was increased to 2.626 at day 203 (Fig 2).

The antibody titre of the final serum collected on day 210 post immunisation that showed an absorbance value (0.423) greater than 2xSDEV (0.184) above the mean of negative control serum (0.229) was 1/8000 as defined by antibody ELISA against purified camel IgG (Fig 3).

Purification of IgG

Purified camel IgG antibody concentration eluted from protein A affinity column was 3.6 mg/ml when measured by QuantiProTM BCA Assay kit (Sigma Chemical Company, UK).

Reactivity of anti-camel IgG raised in rabbits

The ELISA absorbance values of the dialysed rabbit IgG decreased with increasing dilutions of the antibody when titrated against purified camel IgG. An absorbance of 3.189 at a dilution of 1/100 was seen in this IgG, which decreased to 0.708 at a dilution of 1/3200 (Fig 4) but still more than 2xSDEV above the mean of the normal rabbit serum.

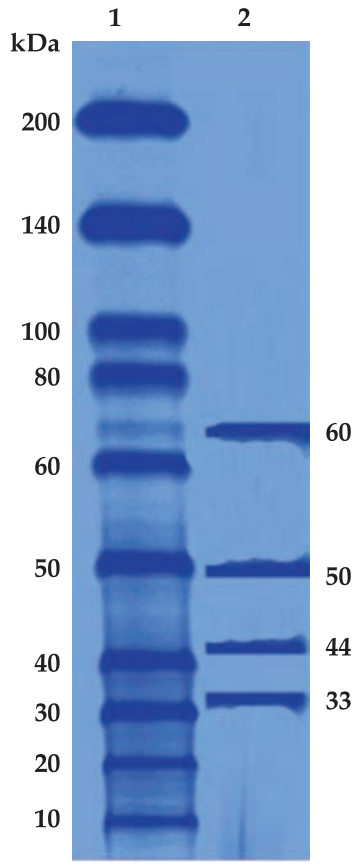

Fig 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis profile of camel immunoglobulin G. Lane 1: Molecular weight marker, Lane 2: Camel immunoglobulin G.

Reactivity of HRP-conjugated IgG by immunoblotting

The conjugated IgG was able to detect three antigenic components in *H. longistipes* soluble extract with molecular weight of 126kDa, 76kDa and 18kDa when tested against serum collected from the naturally infected camel (Fig 5, lane 2). Pre-stained protein ladder of molecular weights ranged from 10-200 kDa was used in the current study (Cell Signaling Danvers, MA, USA).

Discussion

Polyclonal antibodies in hyper-immune serum raised to a single antigen usually contains high proportion of high affinity antibodies with high ability to bind antigen (Greenfield, 2014). Such high affinity and high sensitivity of polyclonal antibodies makes them better reagents in immunoassays. However, the successful production of polyclonal antibodies requires the availability of a highly purified antigen so that the antibody produced will be able to detect the target antigen in complex mixtures. The present study was aimed at producing antiserum to camel IgG and eventually produced anti-camel IgG conjugate to detect *Haemonchus longistipes* antigenic components in infected camel sera by immunoblotting.

A number of IgG purification methods are available of which affinity chromatography with protein A sepharose is the most popular since it produces high yields of pure antibody (Greenfield, 2014). The method is simple, highly efficient (Chan, 1996) and a well-established mean for purification of IgG molecules (Tijssen, 1985; Greenfield, 2014). Although IgM antibodies from some species have been reported to bind to protein A (Tijssen, 1985), the conditions used in the present study, however, were likely to produce predominantly IgG antibodies particularly the high salt buffer conditions under which over 80% of IgM molecules are reported to be removed from the column with washing buffer (Tijssen, 1985). Electrophoretic profile of camel IgG carried out in the present study revealed four bands of molecular weight of 66, 50, 44 and 33 kDa. Similarly, previous workers using 12% homogenous gels under reducing conditions visualised four bands; two bands at 50 and 30 kDa in case of IgG1 while IgG2 and IgG3 produce only one band at 46 and 43

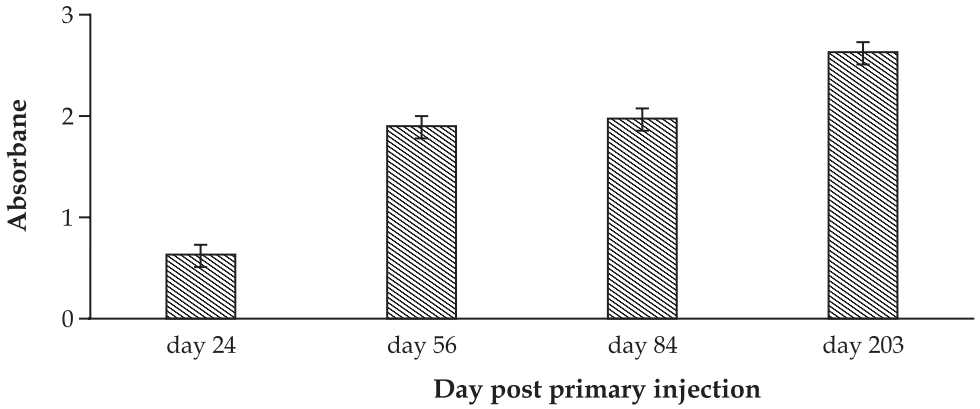

Fig 2. Monitoring antibody response in immunised rabbits

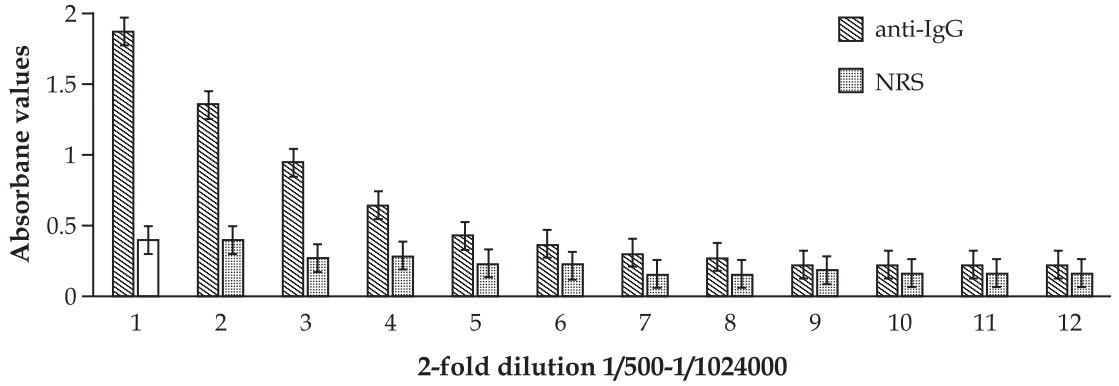

Fig 3. Antibody titre in the final serum collected on day 210 post-primary injection of camel IgG. 1-12 ≡ 2-fold dilution range from 1/500-1/1024000.

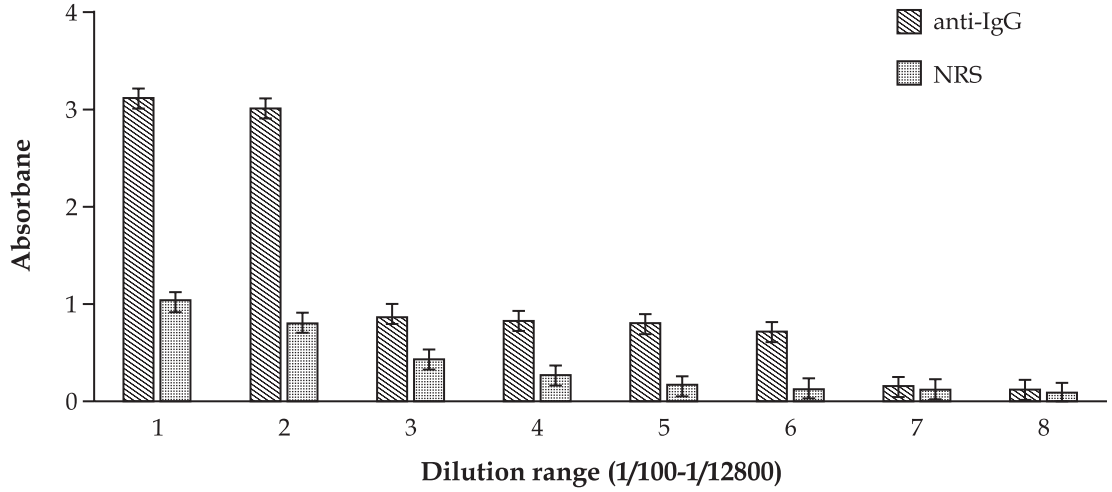

Fig 4. Reactivity of the dialysed rabbit IgG

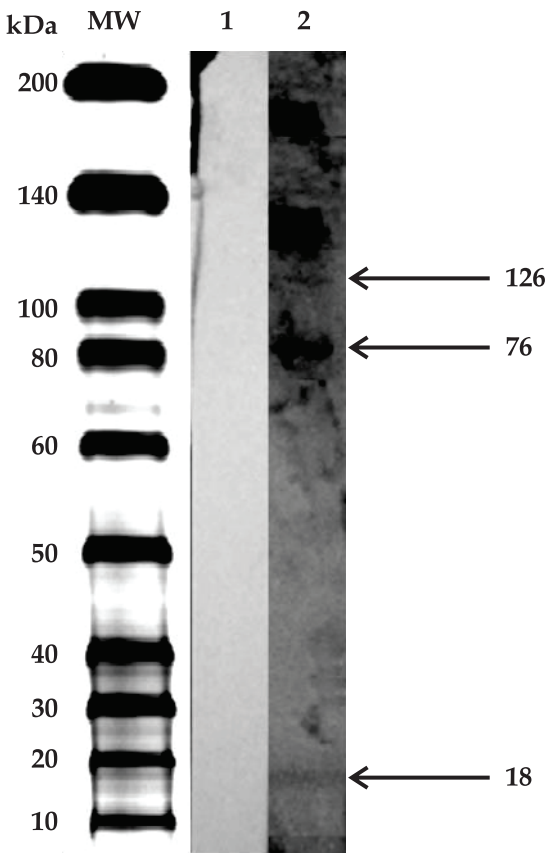

Fig 5. *H. longistipes* proteins identified by rabbit anti-camel IgG in immunoblotting (Lane 1 normal camel serum, lane 2 serum from camel infected with *H. longistipes*).

kDa, respectively (Blanc *et al*, 2008; Mohammadian *et al*, 2010; Shaker and Melake, 2012). Daley *et al* (2005) detected one light chain (22.7 kDa) and one heavy chain (49.4 kDa) for IgG1 and only one dominant heavy chain species of 42.1 kDa for IgG3 and IgG2. While, Abd El Hafez *et al* (2010) using 12% homogenous gels visualised three bands of 52, 46 and 30 kDa. Abdel-Rahman *et al* (2017) detected using 10% homogenous gels detected four bands of 63, 52, 46 and 33 kDa. The camel IgG as 2 bands with molecular masses of 150 and 75 kDa presented by (Khamehchian *et al*, 2014). These differences in bands numbers probably attributed to differences in sample preparation and the type of gels used.

Rabbits are widely used for production of specific antibodies and immunisation of these animals with camel IgG in the present study elicited strong antibody response when tested by antibody-ELISA. An increase in total globulin reaching 4.7 times the amount of globulin at day -1 (pre-immunisation serum) was observed in these animals confirming the usefulness of these animals in production of antibodies.

Immunoassays usually require purified antibodies as the efficiency of these assays is directly dependent on the purity of the antibody (Tijssen, 1985). Enrichment of antibodies is a necessary step achieved by purification and consequently the amount of enzyme needed to conjugate the antibody will be reduced. Anti-camel antibodies produced in the present study were successfully separated from rabbit serum using affinity chromatography. The column eluate in the present study contained the desired antibody as judged by its ability to recognise the camel IgG in ELISA.

The purified IgG antibodies were successfully, labelled with horseradish peroxidase using the modified periodate method (Wilson and Nakane, 1978). This is a well-established method for conjugation of the enzyme, which preserves antibody reactivity by coupling to the carbohydrate portion of the enzyme and antibody molecule, which is not

usually involved in the antigen binding (Liddell and Cryer, 1991). Conjugate produced by this method usually have higher activity than those produced by the two-step glutaraldehyde method with Nygren (1982) reporting five-fold higher detectability in spot-ELISA with periodate conjugates compared with those produced with the two-step glutaraldehyde method.

The IgG after conjugation to horseradish peroxidase retained its activity and was used in western immunoblotting to detect antigenic components of *H. longistipes* in serum of infected camels. The conjugate successfully detected three antigenic components of this parasite in camel sera. The low number of the parasite antigenic components detected in the present study could be attributed to the low number of antigens released during infection and representing excretory-secretory (ES) antigens. ES antigens of similar molecular weights were reported in *H. contortus* (Joshi and Singh, 1999; Bakker *et al,* 2004; Prasad *et al,* 2008) and in other helminth parasites (de Savigny, 1975; Ishiyama *et al,* 2009; Schnyder *et al,* 2011).

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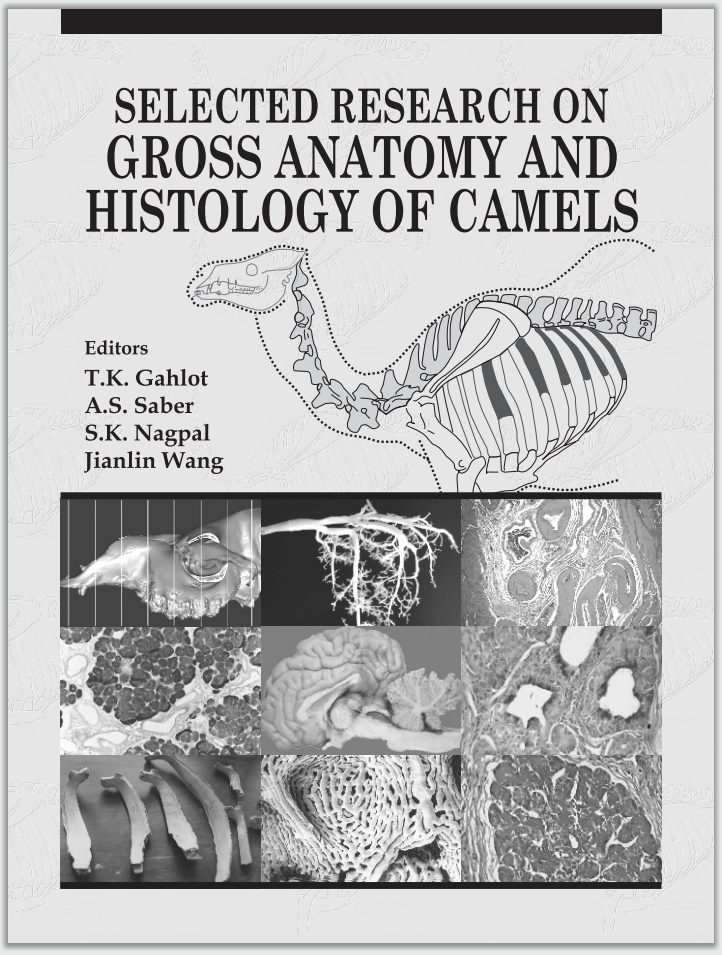

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BIRTH WEIGHT, BODY MEASUREMENTS AND GESTATION LENGTH OF *Tülü* (BACTRIAN X DROMEDARY F1) CALVES

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ABSTRACT

In this study, gestation length (GL), birth weight (BW) and body measurements (BM) of Bactrian x Dromedary F1 calves called *Tülü* born between 2017 and 2020 in Aydın, Turkey were recorded. Fifteen GL records and 25 heads *Tülü* calves' BW and BM were used. The aim was to establish the relationship between BW and BM in order to predict BW from BM. Farm and sex of calf had statistically significant effects (P<0.05) on GL, but the effect of calving number on GL was insignificant (P>0.05). GL of *Tülü* calves were changed between 366 days and 401 days and the mean was found to be 385.27±3.17 days. GL for males (388.7±3.13 days) was 12.6 days longer than those of the female calves (376.1±4.57 days; P<0.05). BW of *Tülü* calves ranged between 26 and 51 kg with an overall mean of 35.99±1.25 kg.

The effects of farm, sire, birth year, calving number and calf sex on BW were found statistically insignificant (P>0.05). However, farm and calving number effects on some BM traits were found statistically significant (P<0.05). Male calves were 0.84 kg heavier than their female counterparts (P>0.05).

It was concluded that male *Tülü* calves have longer gestation length and like other livestock animal, in this study it was found that hearth girth (HG) could be used to estimate BW of *Tülü* calves (R^2=61.16). The higher correlation coefficient found between BW and HG (r=0.782) also supports this idea.

Key words: Birth weight, gestation length, heart girth, stepwise-regression, *Tülü* camel

Turkish camel population had a dramatic decrease by 97% between 1960 and 2000 (Faye, 2020), thereafter increased for the last twenty years, mainly due to the growing popularity of camel wrestling. The people in communities where the racing and wrestling is taking place, aspire to have a camel because it brings a social recognition (Koç and Atasever, 2016). It is believed in the country that a good wrestling camel should be an F1 male offspring produced by crossing Bactrian sire and dromedary dam, named *Tülü* in Turkish (Dioli, 2020).

In recent years a lot of male *Tülü* have been brought to Turkey for wrestling purposes, especially from Iran. Wrestling camel breeders tend to keep the young male *Tülü* camels together (as a cohort) to give them opportunity for playing each other and thus, stimulating and improving their wrestling styles and games. The wrestling activity is started in these camels when these are 7 years old (Manav *et al,* 2018).

The cameleers in Turkey expect to foresee the quality of the wrestler *Tülü* adult based on the birth weight (BW) and/or body measurements (BM). Front leg length is an important criterion for a good wrestling camel because the front legs play a determinant role during wrestling. A long-term study is required to determine BM and BW for *Tülü* camel to be used as wrestlers.

In the present study, BW and BMs of *Tülü* calves as well as their relationships were recorded. In addition, the effect of gestation length was also assessed. Moreover, the correlations between the traits were determined in addition to the establishment of equation to estimate BW from BMs.

Materials and Methods

Animals and Body Measurements

In present study, 25 heads *Tülü* calves born between 2017 and 2020 in four different farms in Aydın, Turkey were used to record BW and BM, 24 hours after birth. BW was taken with a scale weighs up to 2000 kg with an accuracy of 0.5 kg and BMs were taken with a measuring meter with a spirit level and with a 30 cm ruler. The gestation length (GL) was also estimated from the natural mating of 15 heads camels.

BM taken from the calves are described below (Fig 1):

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A-B is wither height (WH): Height of the highest point of the spine (*Processus spinalis*) from the ground,

C-D is rump height (RH): The height of the highest point of the sacrum (the line joining Tuber coxae) from the ground,

E-F is abdominal height (AH): Height of the last back vertebrae from the ground,

K is abdominal girth (AG): The circumference of the last dorsal spine protrusion from under the abdomen,

G-H is body length (BL): The distance between the tip of the shoulder (Tuberculum majus humeri) and the rump of the seat (Tuber ischci),

O-G is neck length (NL): The distance between the lower part of the head and the chest,

J is heart girth (HG): The circumference of the chest bone measured from the back of the forelimbs and under the back and abdomen,

G-L is arm length (AL): The height of the point where the humerus bone meets the scapula,

M-N is tail length (TL): The distance between the point where the tail connects to the body and the tip,

PR is shoulder width (SW): Distance between two shoulder ends,

S-T is rump width (RW): Distance between the tuber ischi's.

Housing and Feeding

All the animals were kept in the barn in single or group paddocks in whole year. After the birth, *Tülü* calves were allowed to suckle their mothers, and the weaning age was very late, about 12 months or more. In two farms the camel milk was sold and calves were allowed to stay and suckle its mother for about 1-1.5 months after birth and the dams were not milked in this period.

When the calves were about 2-3 months old, farmers gave clover hay and concentrate (calf starter used for cattle calf) to them. The calves had also opportunity to drink fresh water *ad libs*. The lactating camels were also fed with roughage, like clover hay and wheat or barley straw and concentrate produced for dairy cattle. While some farms were harvesting alfalfa and withering in the sun before giving it to animals, in some cases in the spring, the grass on the edges of the fields was cut and distributed to the animals.

Statistical Analysis

The differences between the means of the fixed factor levels were taken into account to be statistically significant at $P<0.05$ (2-tailed) based on Tukey's adjustment type I error rate.

Statistical model used for the analysis of GL data is given in Equation I as follow:

$$y_{ijkl}=\mu+a_i+b_j+c_k+e_{ijkl} \quad \text{(I)}$$

where μ is the overall mean, y_{ijkl} of the gestation length, a_i the farm group effects (i=1 and 2), b_j the calving order effects (j=1 and ≥2), c_k the effect of sex (k=Male and Female), and e_{ijkl} the residual random errors.

Statistical model used for the analysis of BW and BMs data is given in Equation II as follow:

$$y_{ijklmn}=\mu+a_i+b_j+c_k+d_l+f_m+e_{ijklmn} \quad \text{(II)}$$

where μ is the overall mean, y_{ijklmn} of the BW or BMs, a_i the farm group effects, (i=1 and 2), b_j the sire effects (j=Cafer Buhur and Deli Buhur), c_k the birth year

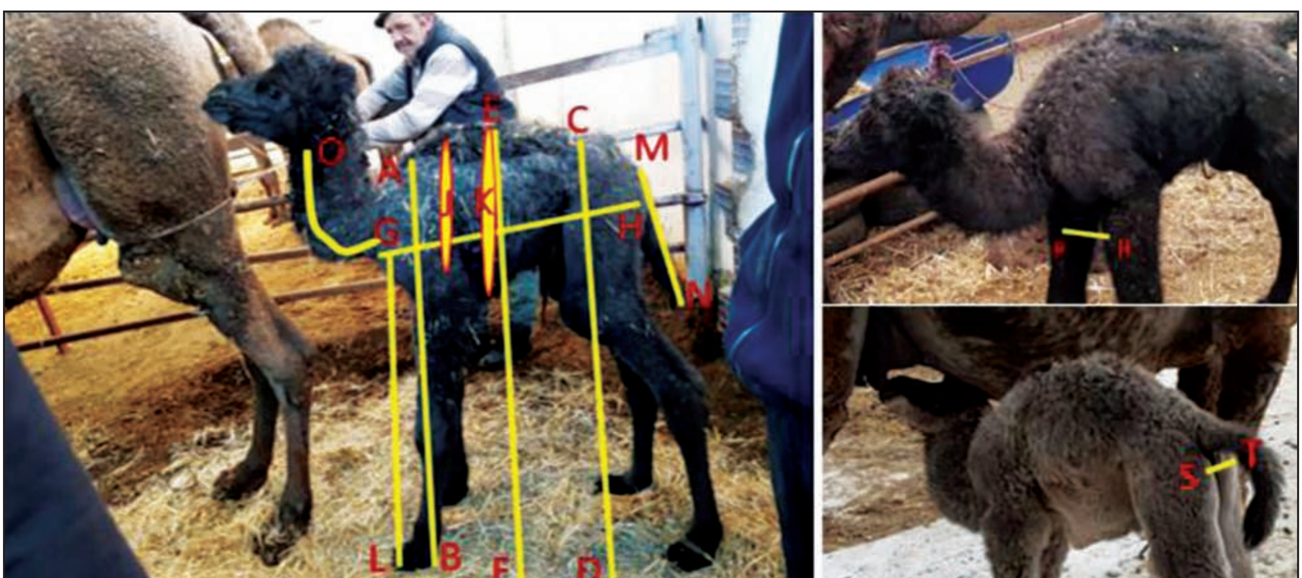

Fig 1. Body measures of *Tülü* calf.

effects (k=2017, 2018, 2019 and 2020), d_l the calving order effects (l=1 and ≥2), f_m the effect of sex (m=Male and Female), and e_{ijklmn} the residual random errors.

The Pearson correlation was used to assess the relationships between BW and the body measurements. The software used for statistical analyses was SAS (1999). In addition, equations were developed by using stepwise-regression procedure in MINITAB 13.0 for the estimation of BW from BMs.

Results

Gestation Length (GL)

The GL varied from 366 to 401 days with a mean value of 385.27±3.17 days. Farm group (P<0.05) and sex of calf (P<0.05) had significant effect on GL, contrary to calving number which was non-significant (Table 1). GL means for first and second farm

Table 1. Gestation length (day) of camel cows

Factor	N	Mean	Min	Max
Farm group		*		
1	8	390.7±3.87	383	399
2	7	374.2±4.68	366	401
Calving Order		NS		
1	7	379.4±4.68	377	399
≥2	8	385.4±3.87	366	401
Sex of calf		*		
Male	10	388.7±3.13	372	401
Female	5	376.1±4.57	366	392
Overall	**15**	**385.27±3.17**	**366**	**401**

NS: not significant, *: Significant for P<0.05

Table 2. Birth weight and body measurements of *Tülü* (Bactrian x Dromedary F1) calves

Factor	n	BW, kg	WH, cm	RH, cm	AH, cm	AG, cm	BL, cm
Farm		NS	NS	*	*	NS	NS
1	15	36.34±1.68	105.87±1.30	103.20±1.11	108.13±1.29	78.33±1.86	59.67±0.96
2	10	35.22±1.93	101.20±2.34	97.45±2.48	102.20±2.54	79.70±2.02	60.40±1.43
Sire		NS	NS	NS	NS	NS	NS
Cafer Buhur	6	32.85±1.10	101.67±1.15	99.17±2.07	102.17±1.45	73.33±2.74	57.50±1.28
Deli Buhur	19	36.85±1.55	104.74±1.62	101.45±1.58	106.89±1.69	80.63±1.36	60.74±0.91
Birth year		NS	NS	NS	NS	NS	NS
2017	8	36.33±2.61	102.38±2.21	99.06±2.56	103.13±2.14	77.00±2.01	59.38±1.22
2018	5	34.14±1.78	103.80±1.66	101.60±2.11	105.20±1.96	78.40±2.77	59.40±2.38
2019	5	36.60±2.87	107.80±2.69	102.80±2.35	107.80±2.96	77.60±0.93	60.80±1.89
2020	7	36.14±2.69	103.29±3.11	101.14±3.08	107.71±3.54	82.29±2.70	60.43±1.81
Calving order		NS	NS	NS	NS	*	*
1≥	10	33.64±1.59	100.70±2.31	97.45±2.52	101.90±2.58	74.90±2.07	57.60±1.09
2	15	37.39±1.72	106.20±1.23	103.20±1.07	108.33±1.18	81.53±1.47	61.67±0.89
Sex		NS	NS	NS	NS	NS	NS
M	15	36.23±1.82	102.93±1.95	99.43±2.00	104.33±2.01	77.80±1.93	59.67±1.11
F	10	35.39±1.60	105.60±1.25	103.10±1.02	107.90±1.56	80.50±1.75	60.40±1.17
Overall	25	35.99±1.25	104.00±1.28	100.90±1.30	105.76±1.38	78.88±1.36	59.96±0.80

NS: not significant, *: Significant for P<0.05; BW: Birth weight, WH: Wither height, RH. Rump height, AH. Abdominal height, AG: Abdominal girth, BL: Body length

Table 2. Continued

Factor	n	NL, cm	HG, cm	SW, cm	AL, cm	RW, cm	TL, cm
Farm group		NS	NS	NS	NS	NS	NS
1	15	40.07±1.45	80.40±1.56	13.33±0.72	88.10±1.38	9.80±0.30	31.53±0.79
2	10	37.50±1.49	76.70±1.80	11.40±0.45	84.20±2.71	8.90±0.53	31.50±1.16
Sire		NS	NS	NS	NS	NS	NS
Cafer Buhur	6	35.50±1.38	77.67±1.58	12.00±1.06	82.25±1.30	9.50±0.43	30.17±1.19
Deli Buhur	19	39.63±1.30	80.11±1.56	12.74±0.58	87.89±1.67	9.42±0.35	31.95±0.76
Birth year		NS	NS	NS	NS	NS	NS
2017	8	35.63±1.34	78.38±2.15	11.63±0.93	83.44±2.61	8.62±0.46	30.63±0.93
2018	5	35.40±1.44	78.40±1.50	12.20±0.80	86.20±1.83	9.20±0.49	30.20±1.44
2019	5	38.40±2.23	79.40±2.77	15.40±1.20	88.40±2.04	9.80±0.80	30.60±0.93
2020	7	44.57±1.57	81.71±3.18	11.86±0.46	89.00±3.38	10.29±0.42	34.14±1.18
Calving order		NS	NS	NS	*	*	NS
1	10	37.50±1.54	77.10±1.86	11.90±0.51	82.15±2.51	10.00±0.52	30.40±1.06
≥2	15	39.40±1.51	81.13±1.58	13.00±0.76	89.47±1.12	9.07±0.30	32.27±0.79
Sex		NS	NS	NS	NS	NS	NS
M	15	38.53±1.52	79.40±1.87	12.13±0.61	85.43±2.07	9.20±0.38	31.20±0.82
F	10	38.80±1.60	79.70±1.48	13.20±0.85	88.20±1.50	9.80±0.42	32.00±1.09
Overall	**25**	**38.64±1.09**	**79.52±1.25**	**12.56±0.50**	**86.54±1.39**	**9.44±0.28**	**31.52±0.65**

NS: not significant, *: Significant for P<0.05, NL: Neck length, HG: Hearth girth, SW: Shoulder width, AL: Arm length, RW: Rump width, TL: Tail length

groups were 390.7±3.87 days and 374.2±4.68 days, respectively. A difference occurred also according to the sex of the camel calf: GL for *Tülü* calves were 388.7±3.13 and 376.1±4.57 days, respectively for male and female (P< 0.05).

A correlation coefficient was also calculated between GL and BW of *Tülü* calves. A moderate and statistically insignificant correlation (r=0.407; P=0.244) was observed.

Birth Weight (BW) and Body Measurements (BM)

The mean BW in our sample was 35.99±1.25 kg. Except the significant effects of sire on RH and AH (P<0.05) and calving order effects on AG, BL, AL and RW, farm, sire, birth year and sex of calf did not show significant effects on BW and BMs (Table 2). The overall means of WH, RH, AH, AG, BL, NL, HG, SW, AL, RW and TL were, 104.00±1.28 cm, 100.90±1.30 cm, 105.76±1.38 cm, 78.88±1.36 cm, 59.96±0.80 cm, 38.64±1.09 cm, 79.52±1.25 cm, 12.56±0.50 cm, 86.54±1.39 cm, 9.44±0.28 cm and 31.52±0.65 cm, respectively.

Estimating Birth Weight (BW) from Body Measurements (BMs)

BW can be estimated from BMs. The equations were reported in Table 3. The stepwise-regression analysis gave two equations with high R^2 values. In the first equation, BW of *Tülü* calves was estimated by using only HG (R^2=61.16). It means that by using only HG, the BW of *Tülü* calves can be estimated with 61.16% accuracy. In the second equation, in addition to HG, SW was also used and R^2 increased slightly for reaching 66.89%. It means that by using HG and SW measures, the BW of *Tülü* calves can be estimated with the accuracy rate over 60%. The addition of other measurements did not improve the prediction.

Table 3. Equations developed to estimate birth weight from body measurements in *Tülü* (Bactrian x Dromedary F1) calves

	Equations	R^2
1	= -26.33+0.78*HG	61.16
2	= -25.53+0.87*HG-0.64*SW	66.89

HG: Heart girth, SW: Shoulder width

Correlations (Table 5)

The phenotypic correlations of BW with BMs were moderate to high, positive and statistically significant, except small positive correlations with SW and RW. The highest positive correlation of BW was with HG (r=0.782; P<0.01). BW was also highly correlated with AG (r=0.607; P<0.01) and NL (r=0.513; P<0.01). None of the negative correlations (SW with AG and TL, then RW with WH and BL) were significant. Other correlations among the BW and BM were positive and generally high.

Unlike other livestock animal, camel has a long neck and it has also high correlations with WH (r=0.488; P<0.01), RH (r=0.554; P<0.01), AH (r=0.663; P<0.01), HG (r=0.667; P<0.01), AL (r=0.562; P<0.01), RW (r=0.474; P<0.05) and TL (r=0.602; P<0.01).

Discussion

Gestation length

Like other livestock species, GL for male *Tülü* calves appeared longer than those of females. Such difference was already observed for long time in dromedary (Agarwal and Khanna, 1993; Al Mutairi *et al,* 2010; Nagy and Juhasz, 2019) and in Bactrian camel (Chen and Yuen, 1984). However, such observations are not constant (Tibary and Anouassi, 1997). For example, unlike this study, Al Mutairi *et al* (2010) reported higher GL for female calves than male in Saudi camels.

Similar to Nagy and Juhasz (2019), the multiparous camels had higher GL than primiparous camels in our study. However, the difference observed in our study (6.0 days) was higher than the difference reported in UAE camel (Nagy and Juhasz, 2019).

With a mean GL of 384.5 ±0.17 days in UAE dromedary camels, Nagy and Juhasz (2019) have assessed the decisive effect of environmental conditions (i.e. photoperiod) on reproductive parameters. With an average of 377.5 days in Saudi camels, Al Mutairi *et al* (2010) reported that season and year of calving had also significant effects on GL, but the effect of parity and sex of calf was not affecting GL significantly. The correlation between GL and BW found for *Tülü* calves in our study was higher than the correlation found for UAE dromedary camels by Bene *et al* (2020) which appeared very weak (r=0.14; P<0.01).

Birth weight

The weight and BMs of livestock animals at birth varies in relation to the breed and the average dam body weight. Since BW of the offspring affect the ease of birth and consequently the survival of the calf, it is therefore considered vital as a management tool.

There are many other factors affecting BW and BM at birth in camel, like genetic factors, age, weight, parity, health and nutrition status of the dam, calf sex, season and geographical region (Koç *et al,* 2018;

Harmas *et al,* 1990; Al Mutairi, 1999). Burger *et al* (2019) reported that season and mother camel (not the breed) play an important role in the variation of gestation length and Birth Weight of the calf.

A higher influence of environment, management, feeding and intrauterine raring capacity of dam on BW than the hereditary growth potential was reported by Bene *et al* (2020).

BW mean found for *Tülü* calves in our study was close to that reported for dromedary camels in some previous studies (Harmas *et al,* 1990; Bakheit *et al,* 2009). However, with a mean value of 35.99 kg, *Tülü* camel calves BW in our sample was slightly higher than those of dromedary calves raised in UAE, the mean BW being 34.5±0.09 kg (Nagy and Juhasz, 2019) and 34.75 ± 5.67 kg (Bene *et al,* 2020). Al Mutairi (2000) reported globally lower BW in African breeds compared to Middle East ecotypes, especially in Saudi Arabia. Indeed, lower BW were reported for the newborn camels in African countries: the mean BW of the newborn dromedary camels were 25.8 in Tunisia and 30.9 kg in Kenya (Burgemeister, 1975; Hertrampf, 2004), or 27 kg in Somalia (Ouda, 1995) and Tunisia according to other reference (Hammadi *et al,* 2001). At reverse, higher BW (39 kg on average) was reported for Indian camel (Bissa, 1996). The same author (Bissa, 2002) reported that BW ranged between 26 and 51 kg in Indian dromedary camel ecotypes. Like values reported by this last author, BW of *Tülü* calves in Turkey was also varied between 26 and 51 kg. However, Bene *et al* (2020) reported surprisingly wider range (10-64 kg) for UAE dromedary camels. Indeed, it is generally admitted that below 25 kg, the probability of survival is very low (Tibary and Anouassi, 1997).

However, the birth weight observed in our sample appeared lower than records generally published for hybrid calves. According to Dioli (2020), Hybrids F1 have an average higher birth weight (45.4±0.842.1kg) than both the calves of Asian dromedaries (Nagy and Juhász, 2019) and of Bactrian camels (34.55±7.17kg according to Zhao *et al*, 2000).

Regarding the sex effect, BW of male *Tülü* calves in our study was 0.84 kg higher than those of female and this result agreed with those of the literature (Al Mutairi, 1999; Bakheit *et al,* 2009). Al Mutairi (1999) reported that the mean BW in male and female calves were 37.45 ± 0.55 kg and 37.27 ± 0.41 kg, respectively. Bakheit *et al* (2009) reported that in Sudanese camel, BW of male calves (39 ± 0.31 kg) was higher than that of female calves (36 ± 0.34 kg). Harmas *et al* (1990) stated that the effect of calf sex on BW is significant, indicating that BW of males was 35.0 ± 0.95 kg and that of females was 34.1 ± 0.46 kg.

The higher birth weight of male calves can be attributed to the developmental factors of males and it can also be in relationship with the longer stay in the dam's uterus than females. This idea is supported by the positive correlation coefficient (r=0.407; P>0.05) determined between GL and BW of *Tülü* calves in this study.

It should be emphasised that maternal uterine conditions have also a significant effect on BW of camel (Bene *et al,* 2020). Twining in camel is very rare (Merkt *et al,* 1990) and due to insufficient nutrition and management, death rates in camel calves sometimes increases to 30-50% (Koç *et al,* 2016), especially in camel with very low BW.

In the late stages of pregnancy, maternal malnutrition causes decrease in the weight gain of the foetus, while the death of the foetus and abortions may also occur depending on the severity of malnutrition (Kadim and Mahgoub, 2013). In different farms of Ethiopia, it was stated that the range of mortality rate in camel calves was 14.9-20.3%, due to insufficient management and nutritional factors (Megersa *et al,* 2008).

In contrast to Al Mutairi (1999), mother age did not have a significant effect on BW of *Tülü* calves in our study. However the measures of AG, BL and AL in *Tülü* calves were lower for the first calving order than those of the second and higher calving order. It was stated that there was a positive correlation between the body weight of the mother and BW of the calf and that the geographical region has also an effect on BW besides the genetic factors (Kadim and Mahgoub, 2013). A high correlation coefficient (r=0.87) between maternal age and calf birth weight was calculated for Saudi camels by Al Mutairi (1999).

The heritability of camel BW was high, and its variation was due to mother (20%), foetus (17%), parity (7%), nutrition (6%), sex (2%) and mother age (1%) (Hansard and Berry, 1969).

Prediction of BW with BM

Like other livestock species, HG could be used in camel to estimate the BW (Table 4). In Holstein-Friesian bulls for example, live weight was estimated with 88.02% accuracy rate (Koç and Akman, 2007). In adult camel, HG was used to estimate BW in the equation proposed by Boué (1949) in Algeria, Graber (1966) in Chad, Field (1979) in Kenya or Bucci *et al* (1984) in Egypt. In his recent comparative study

regarding the accuracy of the different equations published on camel, Boujenane (2019) stated that the formula of Field (1979), where estimated weight (kg) = 6.46*10-7*(WH+HG+AG)3.17, appears to be the best choice. Ihuthia *et al* (2010) estimated live weight of camel from the measures of AG, HG and AH, and Kuria *et al* (2007) reported that the combined effects of HG, AG and WH on body weight were higher than the other individual variables and combination of BMs in camel.

Because of using BW and BM of calves at birth, accepting the formulae given in Table 3 to estimate camel live weight at any age would be giving values with high error. One of the main differences regarding the prediction of live weight from body measurements between the camel calf and adult is the hump. In adult camel, the presence of a big hump has a big influence on the live weight. In our study, because of the hump of calf was not developed yet, its influence is low compared to HG.

In our equation to estimate the weight, SW, NL and AG were also important traits. Besides HG, Koç *et al* (2018) reported AL was also important trait to estimate BW from BM in *Tülü*. Due to having a long neck, in live weight estimation of camel, NL should be one measurement in relationship with the live weight estimation.

The equations developed in our study from 25 heads calves are valid for the measurements between the smallest and the maximum values of the BW and BM recorded. In case of values below the minimum specific or above the higher one, the reliability of the estimate would be reduced. Devore and Pack (1993) described such situations as "danger of extrapolation".

Table 4. Estimated birth weight (BW) based on heart girth (HG) measurements in *Tülü* (Bactrian x Dromedary F1) calves

HG, cm	BW, kg		HG, cm	BW, kg		HG, cm	BW, kg
65	24.4		77	33.7		89	43.1
66	25.2		78	34.5		90	43.9
67	25.9		79	35.3		91	44.7
68	26.7		80	36.1		92	45.4
69	27.5		81	36.9		93	46.2
70	28.3		82	37.6		94	47.0
71	29.1		83	38.4		95	47.8
72	29.8		84	39.2		96	48.6
73	30.6		85	40.0		97	49.3
74	31.4		86	40.8		98	50.1
75	32.2		87	41.5		99	50.9
76	33.0		88	42.3		100	51.7

Correlations

Positive correlations were widely observed between the different measurements. That is a common feature due to the allometric development of body in all species including camel as it was observed in many studies aiming to identify different camel phenotypes based on body measurements (Kamili *et al,* 2006; Chniter *et al,* 2009; Abdallah and Faye, 2012; Oulad-Belkhir *et al,* 2013, Legesse *et al,* 2018; Diop *et al,* 2020).

Conclusion

GL and BW in *Tülü* camel were highly variable under the effect of classical factors as sex, or mother parity. It was possible to predict the birth weight

Table 5. Correlation between birth weight and body measurements in *Tülü* (Bactrian x Dromedary F1) camel

	BW	WH	RH	AH	AG	BL	NL	HG	SW	AL	RW
WH	0.576**										
RH	0.538**	0.911**									
AH	0.583**	0.905**	0.939**								
AG	0.607**	0.379	0.315	0.470*							
BL	0.468*	0.588**	0.577**	0.559**	0.451*						
NL	0.513**	0.488*	0.554**	0.663**	0.443*	0.431*					
HG	0.782**	0.717**	0.643**	0.739**	0.536**	0.408*	0.667**				
SW	0.053	0.498**	0.349	0.392*	-0.111	0.136	0.180	0.355			
AL	0.486*	0.773**	0.815**	0.830**	0.517**	0.599**	0.562**	0.541**	0.127		
RW	0.086	-0.009	0.121	0.114	0.010	-0.203	0.474*	0.100	0.057	0.041	
TL	0.357	0.228	0.332	0.249	0.475*	0.375	0.602**	0.415*	-0.181	0.478*	0.257

*: Significant for P<0.05; **: Significant for P<0.01; BW: Birth weight, WH: Wither height, RH: Rump height, AH: Abdominal height, AG: Abdominal girth, BL: Body length, NL: Neck length, HG: Heart girth, SW: Shoulder width, AL: Arm length, RW: Rump width, TL: Tail length

from body measurements, especially HG with a good accuracy. The mean BW of *Tülü* calf, despite the expected heterosis effect, did not appear exceptional even if it was higher than camel calves from African ecotypes. However, it represents a good starting point to reach the expected weight for a wrestling adult camel. For that, the farmers have to pay special attention to the growth during the first few months of the life of their camel calves.

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GROSS AND MORPHOMETRICAL STUDIES ON DIFFERENT CERVICAL VERTEBRAE IN DROMEDARY CAMELS

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ABSTRACT

The gross anatomical and morphometrical observations of cervical vertebrae of dromedary camels showed many differential features than other domestic animals. The cervical region of camel was made up of seven typical and atypical irregular bones. Longer neck region contributed 46.73% in respect to whole vertebral column. The foramen transversarium was observed in whole cervicals except C7. The length of neural canal was maximum in C2 and minimum in C7. The height of neural canal was greatest in C1 and C7 and least in C2. The Mid-cervical having similar neural canal length but width increased progressively. The atlas having lowest TCV (8.42%) and TVC (3.93%), while C2 contributed highest in TCV (17.78%) and TVC (8.31%).

Key words: Atlas, axis, cervical vertebrae, dromedary camel, foramen transversarium, morphology, morphometry

Most of mammals have seven cervical vertebrae (neck bones), including camel, giraffes, bats, whales, and humans. However, long neck in camel and giraffe is due to more length of vertebrae. The necks of two large and long-necked recent mammals, *Giraffa camelopardalis* and Camelus sp., were examined for the reconstruction of the habitual posture of long necked terrestrial vertebrates (Christian, 2002). The special anatomical characters of cervical vertebrae enables the long necked camel to have a better visibility through required movements of the neck and it also helps them to feed on trees and bushes. Some researchers have studied the cervical vertebrae of camels, previously (Sharma *et al*, 2013; Smuts and Bezuidenhout, 1987; Martini *et al*, 2018). Present research was, therefore, aimed for a detailed gross and morphometrical studies of cervical vertebrae in dromedary camels.

Materials and Methods

The cadavers of 6 camels were processed after proper maceration, cleaning, drying and disinfection with the help of hot air oven and all cervical vertebrae viz. C1 to C7 from every camel were collected. Gross and morphometrical observations, i.e. length, width and thickness were recorded with the help of Vernier caliper, metre scale and thread. The mean and standard error of each measurement were calculated by standard method and analysed.

Results and Discussion

The neck in camel was observed quite longer in comparison with other ungulates. The neck has seven cervical vertebrae along with typical and atypical types. In present study, cervical vertebrae has shown 46.73% of contribution in the entire vertebral column whereas Badlangana *et al* (2009) found it as 45-54% in giraffe, 40% in camel, 44% in lama and 27% in sheep and goat. Sharma *et al* (2013) found the length of camel vertebrae about 28% of total dorsal axial length of camel. The C2-C6 vertebrae were presented with the foramen transversarium which was located at the lateral wall of neural canal. Similar types of observations were reported by Badlangana *et al* (2009) in camel and lama and Torres *et al* (1986) in camelids. However, in other domestic animals and giraffe it penetrates the transverse process, (Badlangana *et al,* 2009 and Ghosh, 2018). The foramen transversarium in C7 was not found in present study, but it was found in giraffe (Badlangana *et al,* 2009). The C2 had a maximum and the C7 had a minimum neural canal length. But, the C1 had the second minimum neural canal length among all the cervical vertebrae, nevertheless C1 has shown minimum neural canal length among atypical and C7 has shown minimum among typical of the cervical vertebrae. The maximum height of neural canal was found at the either side of cervical vertebrae viz. C1 and

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C7. Among which the C1 was maximum than C7, along with the minimum height of neural canal was observed at C2 in animals of present study. These observations were very similar with the findings mentioned by Badlangana *et al* (2009) in giraffe, camel and lama. The similar length of neural canal was observed at C3-C5 vertebrae but the width was increasing progressively. The C6 was found shorter in length and largest in width, while C7 was shortest in length among all typical cervical vertebrae in present study, which was in accordance with observations of Grossman (1960) and Smuts and Bezuidenhout (1987) in dromedary (Table 1).

Atlas vertebrae

The maximum length (104.89±0.84 mm) and maximum width (64.41±1.28 mm) observed in atlas in present study was similar to those reported by Sharma *et al* (2013) and Grossman (1960) in camel. The height of neural canal (43.56±0.23 mm) was the maximum in all cervical cases while the length of the neural canal (46.89±0.81 mm) was less among all of the cervical vertebrae except seventh cervical. The contribution of atlas was lowest in length of total cervical vertebrae (TCV) and total vertebral column (TVC), which was calculated as on 8.42% and 3.93%, respectively. The results were in accordance with Torres *et al* (1986) in camelids. The neural ring was elliptical anteriorly and round shaped posteriorly. On the anterior to fovea dentis a rough articulating area was also noticed at floor, possibly to get the sound grip of atlas and axis and balancing of the skull during movement. The intervertebral foramen was present at the lateral wall of neural ring with an anterior and posterior opening. Anterior and posterior adjacent openings were located at neural canal, however, the anterior opening was at its lateral wall and the posterior was found at the mid lateral area. These findings were in accordance with Torres *et al* (1986) seen in camelids. Researchers found that anterior openings had a common passage with intervertebral canal in camels (Smuts and Bezuidenhout, 1987). The cranial opening was larger and the caudal one was smaller, situated in the mid-lateral of the neural ring. The atlas articulated at anterior end with occipital condyle of skull and its posterior end articulated with anterior of axis. Both the division of atlas viz. anterio-dorsoal and anterio-ventral received the occipital condyle and formed atlanto-occipital joint. These findings were in accordance with Ghosh (2018) in domestic animals. The anterio-dorsal articular area had a division resulted into two parts by a large 'C' shaped notch, however, anterio-ventral area had the large 'C' shaped condyles that were forming two parts with sloping downward. Present findings were in accordance to Smuts and Bezuidenhout (1987) in dromedary. Torres *et al* (1986) did not find any notch/groove found between anterio-dorsal articulation area in camelids. The thickness of anterio-dorsal plate, anterio-ventral plate and anterio-lateral thickness was 1.57±0.10 mm, 23.49±1.39 mm and 5.75±0.41 mm, respectively. The metrical findings on anterio-circular diameter of the atlas were 136.36±2.21 mm, which was comparable with the findings of Sharma *et al* (2013). The posterio-dorsal part of bone was observed as a very thin bony plate, while the posterio-ventral part was very thick and these had metrical measurement of 1.6±0.5 mm and 19.13±1.7 mm, respectively. The posterio-lateral thickness was 1.43±0.1 mm. The posterio-circular diameter was 261.61±3.59 mm. The posterior part which receives the anterior articular area of the axis was a smooth area which had a lateral process that curved backward and upward towards the odontoid

Table 1. Morphometrical observations of cervical vertebrae of camels.

Bone		Atlas		Axis		3rd Cervical		4th Cervical		5th Cervical		6th Cervical		7th Cervical	
		Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
Max. length (mm)		104.89±0.84		221.47± 0.59		196.62± 0.74		193.83±0.61		190.57±0.91		175.55±0.66		162.65±0.57	
Max. width (mm)		64.41±1.28		90.64±0.79		97.54±0.65		118.21±0.59		116.08±0.05		177.54±1.25		135.11±0.93	
Neural Canal (mm)	Min. height	29.07±0.71		21.74±0.38		22.50±0.24		24.05±0.68		26.11±0.08		28.14± 0.21		32±0.17	
	Max. height	43.56±0.23		24.26±0.18		25.38±0.42		29.17±0.73		29.51±0.62		31.13±0.51		33.61±0.48	
	Length from inside	46.89±0.81		125.12±0.60		112.11±1.23		101.78±0.75		92.77±0.90		76.21±1.10		41.71±0.43	
% Contribution		TCV*	TVC**	TCV*	TVC**	TCV*	TVC**	TCV*	TVC**	TCV*	TVC**	TCV*	TVC**	TCV*	TVC**
Length		8.42	3.93	17.78	8.31	15.78	7.37	15.56	7.27	15.29	7.15	14.09	6.58	13.05	6.10

* TCV- Total Cervical vertebrae ** TVC- Total Vertebral Column

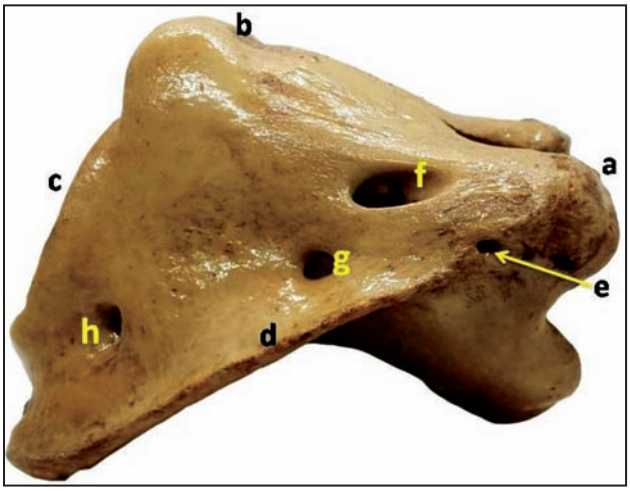

Fig 1. Lateral view of atlas of camel showing (a) Anterior surface, (b) Dorsal surface, (c) Posterior surface, (d) Wing, (e) Accessory alar foramen, (f) Intervertebral canal, (g) Foramen alar, (h) Foramen transversarium.

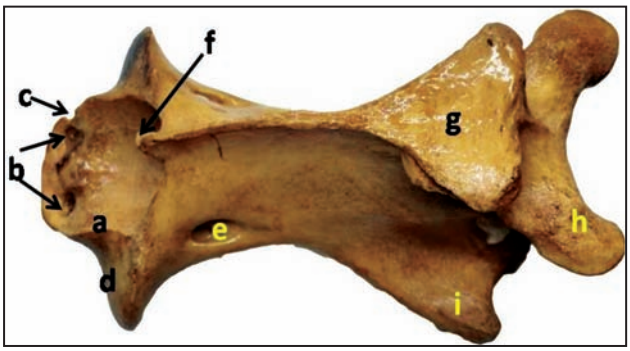

Fig 2. Dorsal view of axis (C2) of camel showing (a) Dens, (b) Lateral depressions of dens, (c) Lateral notch of dens, (d) Anterior articulating surface, (e) Dorsal opening of intervertebral foramen, (g) Bifid dorsal spine, (h) Posterior articulating facets, (i) Wings.

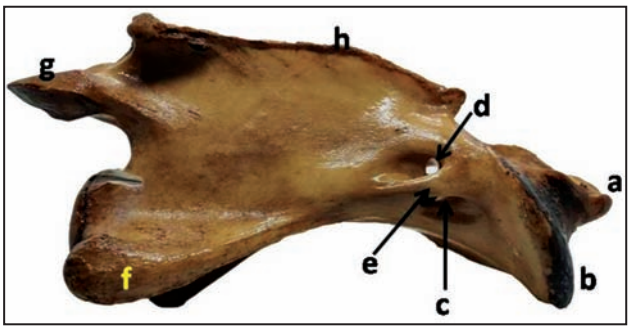

Fig 3. Lateral view of axis (C2) of camel showing (a) Dens, (b) Anterior articulating surface, (c) Ventral opening of intervertebral foramen, (d) Dorsal opening of intervertebral foramen, (e) Bony plate, (f) Wing, (g) Anterior articulating facets, (h) Dorsal spine.

process of axis (C2) vertebrae. These findings were similar to these seen by Torres *et al* (1986) in camelids. The dorsal surface of wings had several perforations after foramen and canals. The smooth dorsal part was

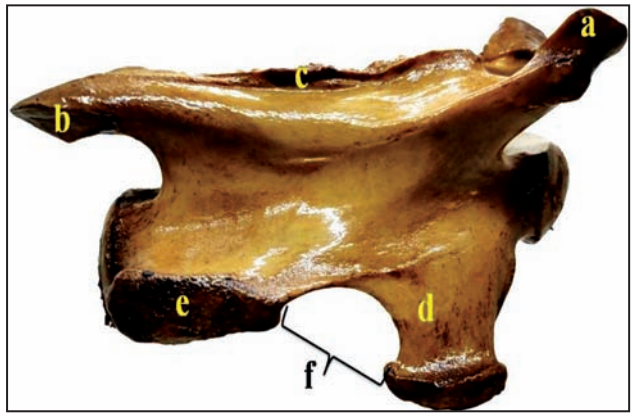

Fig 4. Lateral view of C3 of camel showing (a) Anterior articulating facets, (b) Posterior articulating facets, (c) Dorsal spine (d) Ventral transverse process, (e) Dorsal transverse process, (f) Half moon shaped structure.

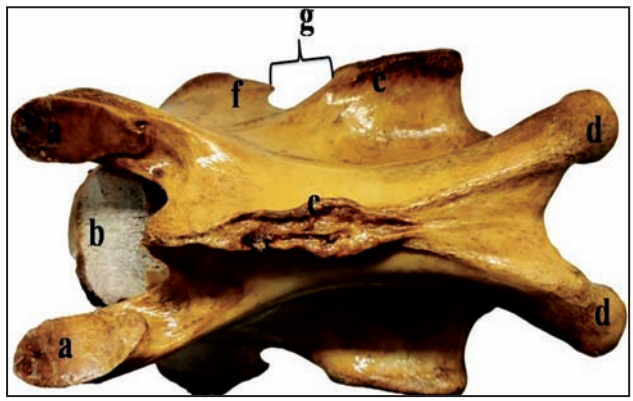

Fig 5. Dorsal view of C4 of camel showing (a) Anterior articulating facets, (b) Body, (c) Dorsal spine, (d) Posterior articulating facets, (e) Dorsal transverse process, (f) Ventral transverse process, (g) Half moon shaped structure.

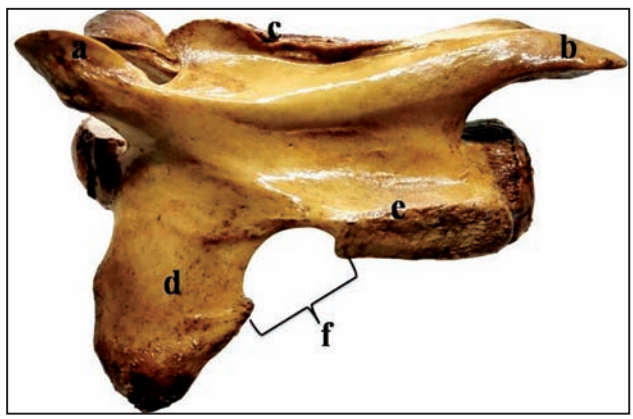

Fig 6. Lateral view of C4 of camel showing (a) Anterior articulating facets, (b) Posterior articulating facets, (c) Dorsal spine, (d) Ventral transverse process, (e) Dorsal transverse process, (f) Half moon shaped structure.

sloping laterally and posteriorly. The thin and convex wings had rough and curved borders. Present results

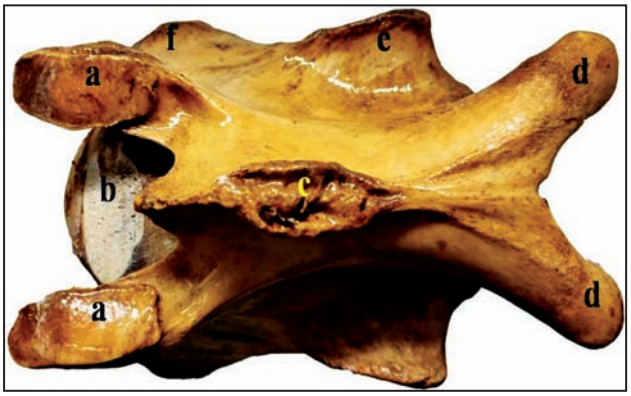

Fig 7. Dorsal view of C4 of camel showing (a) Anterior articulating facets, (b) Body, (c) Dorsal spine, (d) Posterior articulating facets, (e) Dorsal transverse process, (f) Ventral transverse process.

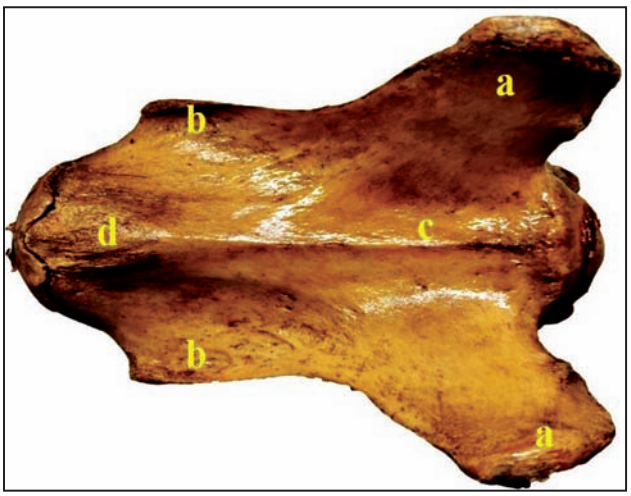

Fig 8. Ventral view of C5 of camel showing (a) Ventral transverse process, (b) Dorsal transverse process, (c) Ventral spine, (d) Ventral tubercle.

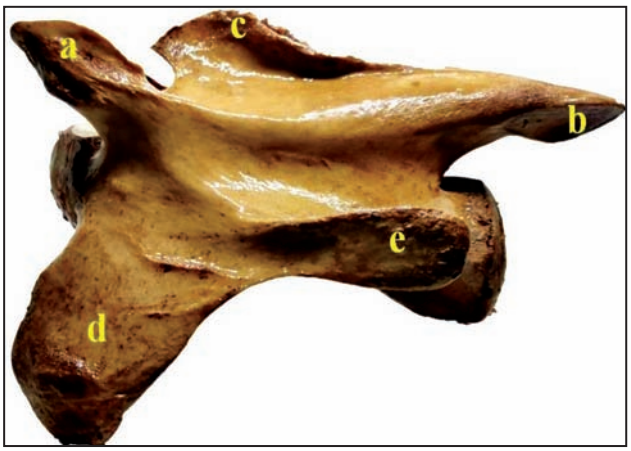

Fig 9. Lateral view of C5 of camel showing (a) Anterior articulating facets, (b) Posterior articulating facets, (c) Dorsal spine, (d) Ventral transverse process, (e) Dorsal transverse process.

Fig 10. Dorsal view of C6 of camel showing (a) Anterior articulating facets, (b) Body, (c) Dorsal spine, (d) Posterior articulating facets.

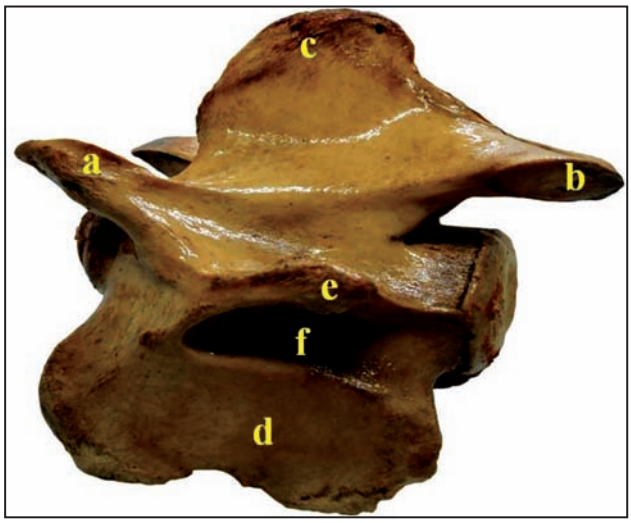

Fig 11. Lateral view of C6 of camel showing (a) Anterior articulating facets, (b) Posterior articulating facets, (c) Dorsal spine, (d) Ventral transverse process, (e) Dorsal transverse process, (f) Shallow fossa.

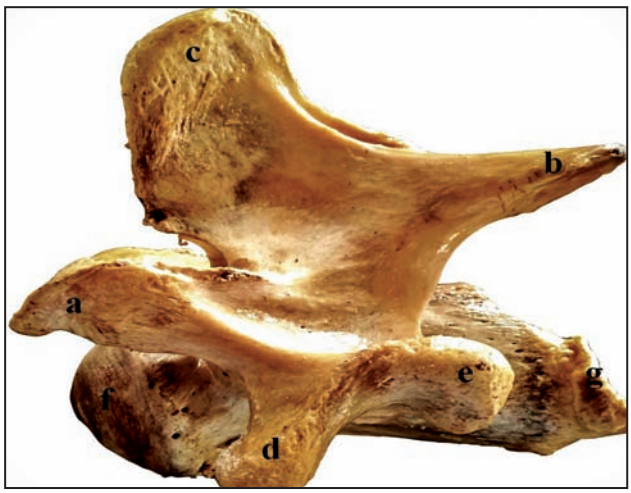

Fig 12. Lateral view of C7 of camel showing (a) Anterior articulating facets, (b) Posterior articulating facets, (c) Dorsal spine, (d) Ventral transverse process, (e) Dorsal transverse process, (f) Anterior part of body, (g) Costal facets.

were in accordance with those of Sharma *et al* (2013) in camels. The posterior part of wings ended in a tubercle which were similar to those seen by Torres *et al* (1986) in camelids.

Ventral surfaces of wings were concave with few rough lines on it. The large depression seen was identified as the fossa atlantis and a large ventral foramen was present near by which was the opening for all four foramen present on the wings of atlas which were in accordance with Grossman (1960). These findings were also in accordance with Ghosh (2018) in domestic animals but Smuts and Bezuidenhout (1987) found double ventral foramen in dromedary. Three foramen and canals were present at anterior part of the wings, while one was at the posterior part. The anterior most foramen was the first that was located on wings with a very small opening, which identified as the accessory alar foramen. The second was the intervertebral larger foramen which was situated towards medial aspect of the wings that had canal connectivity with smaller intervertebral foramen situated at antero-lateral neural canal. The third was the alar foramen which was situated about mid-lateral onto the wings of atlas and it was located just behind the larger intervertebral foramen. Badlangana *et al* (2009) found that dorso-posterior surface had a sloping towards the anterior in giraffe. The dorsal surface of atlas had a rough thin linear abbression initiating from a posteriorly located rough area that reached up to the anterio-dorsal 'C' shaped structure and also the said thin linear abbressions helped to have the two equal halves of dorsal surface. A small 'V' shaped notch was located at anterio-ventral surface which gave the smooth line that traveled up to posterio-ventral surface. The ventral arch was present at the either side lateral to smooth line forming a rough area. Ventral tubercle was observed and these findings were in accordance with these of Torres *et al* (1986) in camelids, but they reported about presence of ventral tubercle. The posterio-ventral portion had slope towards anterio-ventral portion (Fig 1 & Table 1).

Martini *et al* (2018) found the size of the ventral foramen (single opening in the atlantid fossa) as best diagnostic character of atlas, which is so large in Bactrian camels that there is no interspecific over-lap of its diameter in the studied samples. Differences were found in the dorsal foramina; in dromedaries, the cranial (alar) foramina were more distant from each other, had the same distance from the cranial border (at4°), and a greater distance from the caudal (transversal) foramina, suggesting that the last can be closer to the caudal border. In Bactrian camels the vertebral channel was normally higher: this can be seen in the taller cranial and caudal articular opening, and the greater diagonal height of the cranial and caudal articular cavities. All previous analyses (Lesbre, 1903; Wapnish, 1984; Steiger,1990) recognised the diagnostic importance of the ventral foramen in the atlantal fossa. Steiger (1990) suggested that the wings were caudally more developed in Bactrian camels, but we found that these were barely longer in dromedaries. Lesbre (1903) observed that the transversal foramina were closer to the caudal border in dromedaries. The vertebral channel was found dorsoventrally taller in the Bactrian camel.

Axis vertebra (C2)

The C2 was found longest (221.47±0.59 mm) and narrowest (90.64±0.79 mm) of the cervical vertebrae. The smooth, less developed ventral process had a tubercle at its posterior part. The anterior part of the process was in the form of a line that divided the ventral surface into two halves. The odontoid process was tongue like. The contribution of C2 vertebrae was highest in forming the length of total cervical TCV and TVC, which was 17.78% and 8.31%, respectively (Table 1). Intervertebral foramen was located at one-third from anterio-lateral aspect and just behind the anterior opening of the neural canal. It had two large oval shaped openings i.e. dorsal and ventral which were separated by a thin bony plate. The foramen transversarium was observed at anterio-lateral aspect that showed the common opening with intervertebral foramen and connectivity upto the opening at neural ring. The anterior end of the body appeared to be modified in forming the attachment with atlas for which a large 'C'shaped anterior articular surface was there, to form an atlanto-axial joint. The broad body was observed for the articulation along with the rounded neural ring. The dorsal spine was narrow at anterior and middle part while its posterior part was broad, bifurcated, flat, smooth dorsally and rough laterally. Anterior part of the spine was pointed and hanged over the neural ring (Fig 2 & 3). Martini *et al* (2018) found that the axis had classical morphometrical characters, i.e. the common opening of the lateral and the transversal foramina was covered by a bony bridge in dromedaries, but not in Bactrian camels. The bridge was incompletely developed (not closed) in two dromedaries and one Bactrian camel with initial development. Bactrian camels had a greater maximal breadth but at the same time a smaller minimal breadth. The length of both arch and body was greater in dromedaries. Both

Lesbre (1903) and Steiger (1990) saw the presence of a divided lateral foramen in the axis as a reliable distinction.

3rd, 4th and 5th Cervical Vertebrae (C3, C4 and C5)

In their serial placement the bodies of C3, C4 and C5 shorter and wider progressively camel (Fahmy *et al,* 1996). The length and width of C3, C4 and C5 vertebrae mid cervical region plays the balancing role for the neck region of animal. C3 has maximum contribution and C5 was the minimum to the length of vertebrae of mid cervical region.

The supraspinous process which was short and centrally tuberous in the C3, gradually increased in height and length to the C6 with backward inclination. These findings were in accordance with Sharma *et al* (2013) and Fahmy *et al* (1996) in camel. The anterior articulating facets were increased in size, surface area and became less oblique in successive vertebrae. The transverse processes were divided into upper tubercle portion, which projected at right angles, and lower plate-like portion directed outwards, downwards and foreward. The size of the ventral transverse process was increased in successive vertebrae (Sharma *et al,* 2013). In C3 and C4 the dorsal and ventral transverse processes were divided by a half moon shaped structure, but it was absent in C5. The back of this process was pierced by two openings of transverse canals. The anterior opening was present at anterio-lateral aspect of neural canal just below and medial to the anterior articulating facets. The posterior opening was present lateral at two third from the posterior surface of the neural ring. Which were in accordance with Grossman (1960) in camel. The size of foramen transversarium was progressively increased in successive vertebrae. Sharma *et al* (2013) found that it was small and opened into the mid-lateral wall of neural canal. A well-defined infraspinous process was seen on body ventrally which, increased in length and angle of descent. These findings were in accordance with Sharma *et al* (2013) and Grossman (1960) in camel (Fig 4, 5, 6, 7, 8 and 9).

Sixth cervical vertebra (C6)

The supraspinous process of this vertebra was well developed while the infraspinous process was absent. The slope of the spine of the supraspinous process was sharp towards the anterior border. Similar observation were made by Fahmy *et al* (1996); Sharma *et al* (2013) in camel. Length wise C6 was quite similar with C7 but its width was the maximum among all the cervical vertebrae. The anterior opening of foramen transversarium was very large and opened at anterio-lateral aspect of the neural ring. It was located just below the anterior oblique articulating process and dorso-lateral asped of the body. Similar observation were made by Grossman (1960) in camel. Sharma *et al* (2013) were reported that it pierced the anterior part of transverse process in camel. The Neural ring was larger. The Transverse process was divided into dorsal and ventral parts. The dorsal part was situated at posterior aspect while the ventral part was at anterior aspect. The dorsal part was smaller than the similar part of preceding cervical vertebra which was situated dorsally to the ventral transverse process and formed a tubercle at its end. The ventral part of the transverse process was the largest among all the cervical. It covered the ventral part of the body of C6. The ventral part of the transverse process was directed downward and outward in position. There was a shallow fossa present at the lateral surface in between the dorsal and ventral transverse process. Similar observations has were made by with Sharma *et al* (2013) in camel (Fig 10 and 11).

Seventh cervical vertebrae (C7)

C7 shared the characters of both cervical and thoracic segments. The supraspinous process was flat, thin and highest in the length and inclined backward in contrast to ox where it inclined forward. The result were in accordance with Sharma *et al* (2013) in camels and Torres *et al* (1986) in Camelids. Tubercle was present at the anterior and posterior end of the ventral process. The anterior part of the body was short, convex, oval and larger in dimension than preceding cervical. The posterior part was concave from middle and at either side of the body there were two costal facets for the attachment of the first pair of ribs. The transverse process was divided into dorsal and ventral part. The dorsal part was directed backward and outward while the ventral part was directed downward and forward in position. These results were in accordance with Sharma *et al* (2013) in camel. It was larger than all cervicals but without foramen transversarium. Similar results were reported by Sharma *et al* (2013) in camel, and Ghosh (2018) in domestic animals. However, it contradicted the report of Badlangana *et al* (2009) in giraffe where foramen transversarium was present. Torres *et al* (1986) reported that foramen transversarium may or may not be present in C7 of Camelids (Fig 12).

Acknowledgement

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skeletons of different animals while supplying to other educational institutions as the study material.

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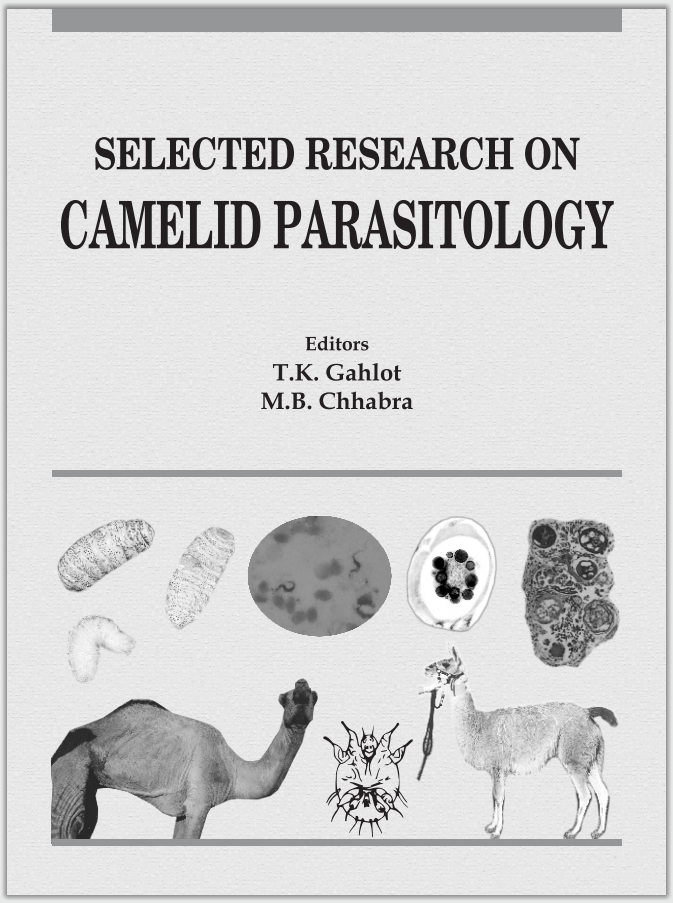

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IMPROVING HEALTH BENEFITS, NUTRITIONAL VALUE AND QUALITY ATTRIBUTES OF LOW FAT ICE MILK MADE FROM CAMEL'S MILK AND DEFATTED CHIA SEEDS FLOUR

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ABSTRACT

Defatted chia seeds flour (DCSF) was prepared and its chemical composition and some functional properties were assessed. Nine chocolate ice milk batches were prepared to study the effect of replacing milk fat with DCSF. Control ice milk contained 4% fat, while the other 8 batches were prepared by replacing 25, 50, 75 and 100% of milk fat with DCSF either at the rate of 50 and 100% of substituted fat. Replacement of milk fat with the same amount of DCSF increased specific gravity, weight per gallon and viscosity, while decreased the freezing point of ice milk mixes. On the other hand substitution of milk fat with DCSF increased melting resistance, total protein, ash content and titratable acidity of ice milk treatments. This increase was more obvious by replacing with fat with the same amount of DCSF than those of ice milk treatments made by replacing milk fat with DCSF at the rate of 50% of substituted fat. Total solids, total protein and ash content and acidity of ice milk treatments did not change significantly during storage period. Replacing milk fat up to 50% with the same amount of DCSF increased the overrun and total scores of organoleptic properties, while increasing the rate of replacement above 50% reduced the overrun and scores of organoleptic properties. Ice milk treatment T_{22} that made with replacing 50% of milk fat with the same amount of DCSF gained the highest total score of organoleptic properties and was most acceptable ice milk treatment. Treatment T_{31} that made with replacing 75% of milk fat with 37.5% of DCSF exhibited higher total score than control ice milk. Therefore, it is possible to milk a good quality low fat ice milk by decreasing 50% of milk fat with 50% of DCSF and / or reducing 75% of milk fat with 37.5% of DCSF.

Key words: Camel's milk, chia seeds, fat replacers, ice milk

Camel's milk is important component in human diet in arid and semiarid zones. There is a growing interest to use camel's milk in the manufacture of many dairy products, because of its crucial health benefits.

Chia (*Salvia hispanica* L.) is an annual herbaceous plant, which has incredible nutritional and functional properties and health benefits it has been cultivated recently in many regions in the world such as Australia, Colombia, Argentina, South-East Asia, Africa, North America and Europe (Ali *et al,* 2012; Romankiewicz *et al,* 2017; Grancieri *et al,* 2017 and Rana 2019). Chia seeds contain higher concentration of protein, dietary fibre, n-3 fatty acids, phenolic compounds, some minerals and vitamins, therefore, it has crucial functional and technological properties such as higher water and oil holding capacity, so it could be used as stabiliser or emulsifier and fat replacer. Chia seeds exhibit tremendous dietary, health and medicinal benefits (Ali *et al,* 2012; Darwish *et al,* 2018; Kulezynski *et al,* 2019; Kwon *et al,* 2019 and Rana 2019). Many studies have been devoted to incorporate chia seed and its products in many food products such as yoghurt, frozen yoghurt, bread, frankfurters, beverage, biscuit, cake, pasta, chips, gravies, soups, salad, cereals, salad dressing, chocolate cakes and biodegradable edible films (Ali *et al,* 2012; Darwish *et al,* 2018; Ikumi *et al,* 2019; Kulegynski *et al,* 2019; Kavon *et al,* 2019 and Rnaa 2019).

There has been substantial interest to develop some dairy products of reduced fat contents to avoid the health problems associated with high fat intake (Williams, 1985; Krauss *et al*, 2000; Niki and Traber, 2012 and Wakai *et al,* 2014).

The objectives of this study were therefore, to assess the chemical composition and some functional

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properties of defatted chia seed flour, evaluate the possibility of making a good quality low fat ice milk using camel's milk with defatted chia seed flour, study the effect of replacing milk fat with defatted chia seed flour on quality of ice milk and to monitor the changes of ice milk quality during storage period.

Materials and Methods

Camel milk was obtained from camel farm at Taif Governorate, Saudi Arabia. Cream was obtained by separating camel's milk in the pilot plant. Non-fat dry milk was obtained from Hoogwegt International BV, Arnhenn, the Netherlands. Stabiliser was gratefully provided by Meer Corporation, North Bergen, NJ, USA. Sucrose, cocoa and corn oil were purchased from the local market.

Preparation of defatted chia seed flour (DCSF)

Chia seeds (*Salvia hispanica* L.) were collected from a farm at Ranyah, Taif, Saudi Arabia and were ground using electric grinder and passed through a 60 mesh sieve. Defatted chia seed flour was obtained (Mansour and Khalil, 2000).

Manufacture of ice cream

Control chocolate ice milk mix was prepared according to Khader *et al* (1992) with the following composition: 4% milk fat, 13% milk solid not fat, 155 sugar, 0.5% stabiliser and 3.0% cocoa. Another 8 ice milk mixes were prepared with the previous composition except that 25, 50, 75 and 100% of milk fat were replaced with defatted chia seeds flour at the rate of 50 and 100% of the substituted fat. All chocolate ice milk mixes were heated at 69°C for 30 min, cooled and then aged over night at 4°C. Ice milk mixes were frozen in ice cream batch freezer (Cattabriga, Bolongia, Italy). The resultant frozen ice milk was packaged in plastic cups and stored in deep freezer at -18°C for 24 hrs. for hardening. All frozen ice milk batches were stored at -20 + 2°C for 10 weeks. All experiments were conducted in 3 replicates.

Physical and chemical analysis

Defatted chia seeds flour was analysed for moisture, total protein, crude fibre, fat and ash according to AOAC (2010), while total phenolic compounds were determined according to Skerget *et al* (2005). Water and oil holding capacities of defatted chia seeds flour were determined as described by Rana (2019). The specific gravity of all ice milk mixes and ice milk samples were determined according to Omar (2014). Weight per gallon of ice milk mixes and ice milk were calculated according to Arbuckle (1986). Freezing point and viscosity of ice milk mixes were determined as described by FAO (1977) and (Morrison and Macjary, 2001), respectively. Melting resistance of ice milk was determined according to Omar (2014). Overrun of ice milk treatments were determined (Arbuckle, 1986). Titratable acidity and fat content of ice milk were determined according to (Ling, 1963). Total solids, ash and total protein were determined (AOAC, 2010).

Sensory evaluation

All ice milk batches were evaluated at zero time and every 2 weeks of storage period using score sheets described by Kebary and Hussein (1997).

Statistical analysis

Completely randomised block design and 2 × 3 factorial design were used to analyse all data. Newman-Keuls Test was used to make the multiple comparisons (Steel and Torrie, 1980) using Costat Program. Significant differences were determined at $p < 0.05$.

Results and Discussion

Physicochemical properties of defatted chia seeds flour

The gross chemical composition of defatted chia seeds flour (DCSF) is presented in table 1. Defatted chia seeds flour contained 38.32% protein, 45.09% crude fibre, 0.76% fat and 8.42% ash content. These values are higher than those reported by Rana (2019). These differences in chemical composition might be due to cultivation region, environmental conditions (climate, availability of nutrient, soil condition, etc.) and genetic factors (Ali *et al,* 2012 and Ikumi *et al,* 2019). Defatted chia seeds flour contained higher concentration of total phenolic compounds (Table 1), therefore, it possess higher antioxidant activity.

Table 1. Mean of physico-chemical analysis of defatted chia seeds flour (DCSF).

Parameter	Mean value
Moisture (%)	7.41 + 0.41
Protein (%)	38.32 + 2.31
Fibre (%)	45.09 + 1.98
Fat (%)	0.76 + 0.02
Ash (%)	8.42 + 0.68
TPC (mg GAE)	0.91 + 0.11
WHC (g/g)	14.19 + 0.39
OHC (g/g)	20.32 + 0.64

WHC: Water holding capacity; OHC: Oil holding capacity; TPC: Total phenolic compounds.

These results are in agreement with those reported by Alfredo *et al* (2009) and Rana (2019).

Defatted chia seeds flour exhibited higher water and oil holding capacities 14.19 and 20.32, respectively. These results were similar to those reported previously (Coorey *et al,* 2012; Olivos-Lugo *et al,* 2010; Rana, 2019). These results might be attributed to the higher fibre and protein content of defatted chia seeds flour as reported by Darwish *et al* (2018) and Alfredo *et al* (2009). The higher water and oil holding capacities of defatted chia seeds flour might enables it to be used as stabiliser emulsifier and fat replacer in the manufacture of many food products (Alfredo *et al,* 2009; Darwish *et al,* 2018; Grancieri *et al,* 2019; Ikumi *et al,* 2019; Rana, 2019).

Ice milk mixes properties

Both specific gravity and weight per gallon of ice milk mixes table 2 followed almost similar trends (El-Kholy, 2018 and Osman *et al,* 2020). Replacement of camel's milk fat with defatted chia seeds flour (DCSF) caused a significant increase of specific gravity and weight per gallon. The increase was more obvious when defatted chia seeds flour was added with the same rate of substituted fat and at the higher rate of replacement (Table 2). This increase was proportional to the rate of substitution. Ice milk mix T_{42} that was made by replacing 100% of milk fat with the same amount of defatted chia seed flour had the highest specific gravity and weight per gallon, while control ice mix exhibited the lowest specific gravity and weight per gallon. These results might be due to the higher specific gravity of defatted chia seeds flour (DCSF) than that of milk fat, which subsequently increased the specific gravity of ice milk mixes. Similar trends were reported by other researchers (Hamed *et al,* 2014; Kamaly *et al,* 2017).

Table 2. Effect of replacing camel's milk fat with defatted chia seeds flour (DCSF) on some properties of low fat ice milk mixes.

Ice mix treatments*	Specific gravity	Weight / gallon (kg)	Freezing point (°C)	Viscosity (cp)
C	1.1129^{F}	4.2134^{F}	-2.19^{A}	238.1^{F}
T_{11}	1.1134^{E}	4.2153^{E}	-2.24^{B}	240.2^{E}
T_{12}	1.1141^{D}	4.2180^{D}	-2.29^{C}	245.6^{D}
T_{21}	1.1143^{D}	4.2187^{D}	-2.33^{D}	246.3^{D}
T_{22}	1.1149^{C}	4.2210^{C}	-2.40^{E}	251.2^{C}
T_{31}	1.1148^{C}	4.2206^{C}	-2.43^{E}	248.6^{C}
T_{32}	1.1156^{B}	4.2237^{B}	-2.50^{F}	256.3^{B}
T_{41}	1.1153^{B}	4.2225^{B}	-2.56^{F}	252.9^{C}
T_{42}	1.1166^{A}	4.2274^{A}	-2.66^{G}	264.2^{A}

Means followed with different letters within each column are not significantly different according to Duncan test (P = 0.05).
*C: the control mix.
T_{11} and T_{12}: Ice mix treatments containing 3% fat with adding 0.5 and 1.0% DCSF.
T_{21} and T_{22}: Ice mix treatments containing 2% fat with adding 1.0 and 2.0% DCSF.
T_{31} and T_{32}: Ice mix treatments containing 1% fat with adding 1.5 and 3.0% DCSF.
T_{41} and T_{42}: Ice mix treatments without fat adding 2.0 and 4.0% DCSF.

Freezing point of low fat ice mixes decreased significantly ($p < 0.05$) by increasing the rate of replacing milk fat, with defatted chia seeds flour (DCSF). Ice milk mix T_{42} that was made by replacing 100% of milk fat with DCSF exhibited the lowest freezing point, while control ice milk mix had the highest freezing point (Table 2). These results could be attributed to the dissolve of some DCSF constituents and / or differences of mixes acidity that help to dissolve some mix constituents which consequently cause the decrease in freezing point of ice milk mix, Kebary (1996) and Khalil and Blassey (2019) reported that when fat was replaced with dissolved substances in ice cream the freezing point decreased. Osman *et al* (2020) also stated that freezing point of ice cream mix was decreased by replacing of milk fat with ingredient containing higher concentration from fibre and mineral, which is similar to defatted chia seeds flour where it contains higher concentration from fibre and minerals (Table 1).

Replacement milk fat with DCSF caused a pronounced increase in the viscosity. There was a positive correlation between the rate of replacing milk fat milk with DCSF and the viscosity of ice milk mixes (Table 2). Replacing milk fat with the same amount from DCSF was more efficient to increase the viscosity of ice milk mixes (Table 2). These results might be due to the higher water holding capacity of DCSF and consequently its higher ability to retain water, which increase the viscosity (Borneo *et al,* 2010; Darwish *et al,* 2018; El-Kholy, 2018; Ervina and Abdilla, 2018; Jain and Rai, 2018; Grancieri *et al,* 2019; Ikumi *et al,* 2019 and Osman *et al,* 2020). Similar results were reported by (Hamed *et al,* 2014 and Kamaly *et al,* 2017) who used inulin as a fat replacer.

Properties of ice milk

Specific gravity and weight per gallon of ice milk treatments followed similar trends in reported by El-Kholy (2018) and (Khalil and Blassey, 2019). Replacement of milk fat with defatted chia seeds flour (DCSF) caused a marked increase of both specific gravity and weight per gallon of ice milk

treatments (Table 3). This increase was proportional to the rate of replacement. The differences among ice milk treatments in both specific gravity and weight per gallon could be attributed to 2 factors, first factor is using DCSF which has higher specific gravity than milk fat and the second factor is the overrun which has negative correlation with specific gravity and weight per gallon (Hamed *et al,* 2014; El-Kholy, 2018; Osman *et al,* 2020). Ice milk treatment T_{42} which made by replacing 100% of milk fat with the same amount of DCSF and had the lowest overrun exhibited the highest specific gravity and weight per gallon, while ice milk treatment C, which made without replacing milk fat exhibited the lowest specific gravity and weight per gallon (Table 3). These results are in agreement with those of others researchers (Hamed *et al,* 2014; Kamaly *et al,* 2017; El-Kholy, 2018; Osman *et al,* 2020) who substituted milk fat with different fat replacers.

Table 3. Effect of replacing camel's milk fat with defatted chia seeds flour (DCSF) on some properties of low fat ice milk.

Ice milk treatments*	Specific gravity	Weight / gallon (kg)	Overrun	Melting resistance		
				First 60 min	Next 30 min	Last 30 min
C	0.6738^{D}	2.5510^{D}	63.45^{C}	30.60^{A}	43.3^{A}	26.1^{F}
T_{11}	0.6785^{D}	2.5688^{D}	65.09^{C}	29.2^{A}	43.6^{A}	27.2^{F}
T_{12}	0.6816^{C}	2.5805^{C}	66.38^{B}	28.3^{B}	41.9^{B}	29.8^{E}
T_{21}	0.6829^{C}	2.5854^{C}	66.09^{B}	28.1^{B}	41.5^{B}	30.4^{E}
T_{22}	0.6883^{C}	2.6059^{C}	68.16^{A}	26.9^{C}	39.7^{C}	33.4^{D}
T_{31}	0.6953^{B}	2.6324^{B}	63.09^{C}	27.5^{C}	39.6^{C}	32.9^{D}
T_{32}	0.6992^{B}	2.6472^{B}	59.13^{E}	25.6^{D}	37.2^{D}	37.2^{B}
T_{41}	0.6936^{B}	2.6259^{B}	61.57^{D}	27.0^{C}	38.9^{C}	34.1^{C}
T_{42}	0.7138^{A}	2.7024^{A}	55.18^{F}	23.1^{E}	36.1^{E}	40.8^{A}

Means followed with different letters within each column are not significantly different according to Duncan test (P = 0.05).

Overrun of ice milk increased significantly by increasing the rate of replacing milk fat with the same amount of DCSF up to 50% which might be due to improving the whipability of ice milk by adding DCSF (Grancieri *et al,* 2019 and Ikumi *et al,* 2019). El-Kholy (2018) reported that the increase of overrun might be due to the interaction between dietary fibre and milk proteins and formation of complex matrix that increase the entrapment of air in ice milk. Increasing the rate of replacing milk fat with the same amount of DCSF above 50% caused a significant ($p < 0.05$) reduction of overrun of ice milk treatments (Table 3). This decrease of overrun might be due to the increase of viscosity that subsequently suppress the ability of ice milk to retain the air in ice milk (Chang and Hartel, 2002; Sofjan and Hartel, 2004). Ice milk treatment (T_{21}, T_{22}, T_{32} and T_{43}) those made by replacing milk fat with the same amount from DCSF were significantly different from corresponding ice milk treatments (T_{11}, T_{21}, T_{31} and T_{41}) those made by adding DCSF at the rate of 50% of substituted fat (Table 3). Similar trends were reported by Hamed *et al* (2014) and Kamaly *et al* (2017).

Replacement of milk fat with DCSF caused an obvious reduction of the rate of melting, which means increasing the melting resistance of ice milk at 60 min and the next 30 min (Table 3). This increase of melting resistance was proportional to the rate of replacing milk fat with DCSF. Ice milk treatment T_{42} that made with replacing 100% of milk fat with the same amount of DCSF exhibited the highest melting resistance while control ice milk exhibited the least melting resistance (Table 3). These results might be due to the higher fibre content of DCSF that increase the viscosity and the higher water holding capacity (Alfredo *et al,* 2009; Darwish *et al,* 2018; Ikumi *et al,* 2019; Rana, 2019), which binds higher amount of water and left lowest amount of free water that can be melted faster than the bound water, therefore increase the melting resistance. These results are in agreement with those of other researchers (Ervina and Abdillah, 2018; Jain and Rai, 2018; El-Kholy, 2018; Aljewicz *et al,* 2020; Kebary *et al,* 2020; Osman *et al,* 2020). Ice milk treatments (T_{12}, T_{22}, T_{32} and T_{42}) made by replacing milk fat with the same amount of DCSF exhibited higher melting resistance than those of corresponding ice milk treatments (T_{11}, T_{21}, T_{31} and T_{41}) which were made by adding DCSF at the rate of 50% of substituted fat (Table 3). These results might be due to the differences in fibre content and water holding capacity. On the other hand, melting resistance of ice milk treatments after the last 30 min followed contradictory trend of those at the first 60 min. These results were in accordance with those reported by Hamed *et al* (2014), Kamaly *et al* (2017) and Kebary *et al* (2020).

Ice milk treatments were significantly ($p < 0.05$) different from each other in titratable acidity, which means replacement of milk fat with DCSF increased significantly ($p < 0.05$) the titratable acidity of the resultant ice milk treatments (Tables 4 and 6). There was positive correlation between the rate of replacement and the titratable acidity of ice milk treatments.

Ice milk treatment T_{42} that made with replacing 100% of milk fat with the same amount of DCSF had

the highest acidity, while control ice milk had the lowest titratable acidity (Tables 4 and 6). Ice milk treatments (T_{12}, T_{22}, T_{32} and T_{42}) those made by replacing milk fat with the same amount from DCSF exhibited higher acidity than corresponding ice milk treatments (T_{11}, T_{21}, T_{31} and T_{41}) those made by replacing milk fat with only 50% of DCSF (Tables 4 and 6). These results could be attributed to the higher protein content of DCSF which increases the buffering capacity and consequently increases the titratable acidity of the resulting ice milk treatments. On the other hand, titratable acidity of all ice milk treatments did not change significantly ($p > 0.05$) during storage period (Tables 4 and 6). These results are in agreement with those reported by Hamed *et al* (2014) and Kebary *et al* (2020).

Ice milk treatments C, T_{12}, T_{22}, T_{32}, and T_{42} were not significantly ($p > 0.05$) different from each other in total solids content, which means replacing of milk fat with the same amount of DCSF did not have significant effect on total solids content of the resultant ice milk treatments (Tables 4 and 6). Similar results were reported by Hamed *et al* (2014) and Kamaly *et al* (2017), who used inulin as a fat replacer. On the other hand, replacement of milk fat with DCSF at the rate of 50% of substituted fat caused a significant reduction of total solids of the resultant ice milk treatments and this decrease of total solids was proportional to the rate of replacement (Tables 4, 6). Total solids content of all ice milk treatments did not change significantly ($p > 0.05$) throughout the frozen storage period (Tables 4, 6). Similar results were reported by Hamed *et al* (2014) and Kebary *et al* (2020).

Fat content of ice milk treatments decreased by increasing the rate of replacing milk fat with DCSF (Tables 4, 6). There was negative correlation between the rate of replacement and the fat content of ice milk treatments. Control ice milk treatment contained the highest fat content, while those treated (T_{41} and T_{42}) and made by replacing 100% of milk fat with DCSF contained the lowest fat content. These results were in agreement with those reported by Hamed *et al* (2014) and Kamaly *et al* (2017).

Replacement of milk fat with DCSF either at the rate of 100% or 50% of substituted fat did not have significant ($p > 0.05$) effect on the fat content of ice milk treatments. Therefore ice milk treatments (T_{12}, T_{22}, T_{32} and T_{42}) did not change significantly ($p > 0.05$) from corresponding ice milk treatments (T_{11}, T_{21}, T_{31} and T_{41}), respectively. Fat content of all ice milk treatments did not change significantly ($p > 0.05$) as storage period proceeded (Tables 4 and 6) (Hamed *et al,* 2014; Kamaly *et al,* 2017; Kebary *et al,* 2020).

Total protein and ash contents of ice milk treatments followed almost similar trends (Tables 4 and 6). Both total protein and ash content of ice milk treatments increased significantly ($p < 0.05$) with replacing milk fat with DCSF added either at the rate of 100% or 50% of substituted fat (Tables 4, 6). This increase was proportional to the rate of replacement (Tables 4 and 6). The increase of protein and ash content of ice milk treatments (T_{12}, T_{22}, T_{32} and T_{42}) those made by replacing milk fat with the same amount of DCSF was more pronounced than those of ice milk treatments (T_{11}, T_{21}, T_{31} and T_{42}) made by replacing milk fat with DCSF @ 50% of substituted fat (Tables 4 and 6). Ice milk treatment (T_{42}) that was made by replacing 100% of milk fat with 100% of DCSF contained the highest total protein and ash contents, while control ice milk treatment contained the lowest total protein and ash contents (Tables 4 and 6). These results might be due to the higher total protein and ash content of DCSF (Alfredo *et al,* 2009;

Table 4. Effect of replacing camel's milk fat with defatted chia seeds flour (DCSF) on the gross composition of low fat camel ice milk during the frozen storage for 10 weeks.

Ice milk Treatments*	Total solids (%)		Fat (%)		Proteins (%)		Ash (%)		Titratable acidity (%)	
	Fresh	10 weeks	Fresh	10 weeks	Fresh	10 weeks	Fresh	10 weeks	Fresh	10 weeks
C	34.25	34.76	3.9	4.0	4.92	4.81	1.08	1.10	0.225	0.230
T_{11}	33.76	34.05	2.9	2.9	5.06	5.09	1.12	1.18	0.231	0.234
T_{12}	34.33	34.51	2.9	2.9	5.19	5.22	1.16	1.19	0.238	0.243
T_{21}	33.28	33.43	2.0	2.0	5.18	5.20	1.16	1.21	0.239	0.242
T_{22}	34.21	34.56	2.0	2.0	5.43	5.50	1.22	1.25	0.248	0.251
T_{31}	22.17	32.41	1.0	1.0	5.36	5.41	1.21	1.26	0.246	0.249
T_{32}	34.31	34.58	1.0	1.1	5.71	5.78	1.28	1.30	0.253	0.256
T_{41}	32.31	32.46	0.1	0.1	5.41	5.46	1.23	1.29	0.249	0.254
T_{42}	34.43	34.66	0.1	0.1	6.09	6.16	1.39	1.42	0.258	0.262

Grancieri *et al,* 2019; Ikumi *et al,* 2019; Rana, 2019). On the other hand, total protein and ash contents of all ice milk treatments did not change significantly ($p > 0.05$) as storage period progressed (Tables 4 and 6). These results are in agreement with reported by other researchers (Hamed *et al,* 2014; Kamaly *et al,* 2017; Kebary *et al,* 2020).

Scores of organoleptic properties (flavour, body and texture, melting quality and colour) of ice milk treatments stored for 10 weeks are presented in Table 5. There were slight differences among ice milk treatments in body and texture and melting quality, while there were no significant ($p > 0.05$) differences in the colour of ice milk treatments. Replacement of milk fat up to 50% with the same amount of DCSF increased the scores of flavour and the total scores and improved the acceptability of ice milk treatments, while increasing the rate of replacement above 50% reduced the scores of flavour and the total scores of organoleptic properties (Tables 5 and 6). Ice milk treatment (T_{22}) that was made by replacing 50% of milk fat with the same amount of DCSF gained the highest total scores of organoleptic properties and was the most acceptable ice milk treatments (Tables 5 and 6). Also ice milk treatment (T_{31}) that was made with replacing 75% of milk fat with DCSF at the rate of 50% of substituted fat gained total scores of organoleptic properties higher than control ice milk, but lower than treatment (T_{22}) (Tables 5 and 6). These results might be due to the higher fibre content of DCSF that provided a uniform smooth body and texture (Soukoulis *et al,* 2009). On the other hand, the total scores of organoleptic properties of all ice milk treatments did not change significantly during storage period up to 8 weeks, then declined up to the end of storage period (Hamed *et al,* 2014; Kebary *et al,* 2020).

Defatted chia seeds flour (DCSF) contained higher concentration of total protein, fibre ash and

Table 5. Effect of replacing camel's milk fat with defatted chia seeds flour (DCSF) on the scores of organoleptic properties of low fat ice milk during the frozen storage for 10 weeks.

Ice milk treatments*	Flavour (45)						Body and texture (35)						Melting quality (10)						Colour (10)						Total score (100)					
	Storage period (weeks)																													
	0	2	4	6	8	10	0	2	4	6	8	10	0	2	4	6	8	10	0	2	4	6	8	10	0	2	4	6	8	10
C	40	40	40	38	38	36	32	32	31	30	30	30	9	9	8	8	7	7	9	9	8	8	8	7	90	90	87	84	83	80
T_{11}	42	42	40	38	38	35	32	32	31	30	30	30	9	9	8	8	8	7	9	9	8	8	8	7	92	92	88	84	84	79
T_{12}	42	42	40	39	37	36	32	32	30	30	29	29	9	9	9	8	8	7	9	8	8	8	8	7	92	91	87	85	82	79
T_{21}	41	41	40	38	36	34	33	32	31	30	28	26	9	9	9	8	8	8	9	8	8	8	7	7	92	91	88	84	81	75
T_{22}	43	42	40	38	35	35	33	33	30	30	30	30	9	9	9	8	8	8	9	8	8	8	7	7	94	91	86	84	80	80
T_{31}	41	40	40	39	36	34	31	33	32	30	30	28	9	9	9	8	8	8	9	9	9	8	8	7	92	90	87	85	82	77
T_{32}	39	38	38	36	34	32	31	30	30	29	30	28	9	9	8	8	8	7	8	8	8	8	7	7	87	85	84	81	79	74
T_{41}	40	40	40	38	36	34	32	31	30	28	28	28	9	9	8	8	8	8	8	8	8	8	8	7	89	88	86	82	82	77
T_{42}	36	36	36	34	31	30	30	28	28	28	27	26	8	8	8	8	7	7	8	8	7	7	7	7	82	80	79	77	72	70

Table 6. Statistical analysis of low fat ice milk properties.

Camel ice milk properties	Means square	Effect of treatments									Means square	Effect of storage (weeks)					
		C	T_{11}	T_{12}	T_{21}	T_{22}	T_{31}	T_{32}	T_{41}	T_{42}		0	2	4	6	8	10
Total solids	5.367*	A	B	A	C	A	D	A	E	A	2.610	A					A
Fat	0.381*	A	B	B	C	C	D	D	E	E	0.870	A					A
Ash	0.071*	F	F	E	E	C	D	B	C	A	0.076	A					A
Proteins	0.547*	G	F	E	E	C	D	B	C	A	0.765	A					A
Titratable acidity	0.077*	E	D	C	C	B	B	AB	B	A	0.861	A					A
Organoleptic properties:																	
Flavour	21.186*	C	B	B	B	A	B	D	C	E	3.214*	A	A	A	A	AB	B
Body and texture	11.125*	A	A	A	A	A	A	A	A	A	0.192*	A	A	A	A	AB	B
Melting quality	1.831	A	A	A	A	A	A	A	B	B	0.056*	A	A	A	A	AB	B
Colour	1.936*	A	A	A	A	A	A	B	B	B	0.139*	A	A	A	A	AB	B
Total score	76.13*	C	B	B	B	A	B	D	D	E	0.831	A	A	A	A	AB	B

Means followed with different letters within each column are not significantly different according to Duncan test ($P = 0.05$).

exhibited higher water and oil holding capacities. Replacement of milk fat with DCSF especially, at the rate of 100% of substituted fat increased the specific gravity, weight per gallon and viscosity, while decreased the freezing point of ice milk mixes. Substitution of milk fat with DCSF caused a significant increase of melting resistance, total protein, ash and acidity of ice milk. Replacement of milk fat up to 50% with the same amount of DCSF increased the overrun and the total scores of organoleptic properties of ice milk, while increasing the rate of replacement above 50% decreased the total scores and the overrun. Therefore, it could be recommended that it is possible to reduce the fat content of ice milk that was prepared by using camel's milk up to 50% with adding the same amount of DCSF, without detrimental effects on the resultant ice milk and / or reducing milk fat up to 75% with adding only 37.5% from DCSF.

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MODERN ADVANCES ON THE DIAGNOSIS OF BOVINE VIRAL DIARRHOEA VIRUS IN CAMELIDS

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ABSTRACT

Bovine viral diarrhoea virus (BVDV) is one of the most leading causes of diarrhoea in many species of animals including camelids. Special attention is being paid to studying various aspects of the BVDV infection, pathogenesis, epidemiology, diagnosis and vaccines in the bovine species. Little is still known about these aspects in camelids especially dromedaries. The development of accurate diagnostic tests is a critical step for the identification of infected animals, especially persistently infected and for the control and eradication of BVDV. Although adaptation of the currently used diagnostic assays for the virus in the bovine and ovine species for the diagnosis of BVDV in the camelids, it is highly recommended to develop novel specific diagnostic assays of the virus in camels. There are several strategies for the diagnosis of BVDV including virus isolation, detection of viral (antigens, antibodies and nucleic acids) and identification of the circulating strains of the virus by doing sequencing and phylogenetic analysis. Identification of the persistently infected animals is one of the main challenges facing the diagnosis and control of the virus. Our main objectives are to highlight the most recent advances in the field of BVDV diagnosis, currently used diagnostic assays and suggested some specific approaches for the diagnosis of BVDV in camelids. Fine-tuning the currently used diagnostic assays and development of other novel specific assays to detect BVDV infection in dromedary camels will have a substantial positive impact on the control of BVDV infections in camels.

Key words: Assays, BVDV, camels, detection, diagnosis, isolation, molecular

The bovine viral diarrhoea virus (BVDV) was previously known as a mucosal disease that is responsible for high economic losses among the affected animals in most countries around the world (Fray *et al,* 2000). BVDV represents one of the common causes of immunosuppression, mucosal disease and reproductive failures among various species of animals such as cattle, sheep, goats and other heterologous species of animals such as camelids (Passler and Walz, 2010).

BVDV is a member of the Pestiviruses causing serious problems in the affected species of animal such as reproductive failure, decrease in milk production and abortion (Ostachuk, 2016, Passler and Walz, 2010). Classification of BVDV based on the ability to grow on cell culture and induce cytopathology revealed two genotypes called BVDV-1 and BVDV-2 (Al-Kubati *et al,* 2021).

There are several approaches for the diagnosis of BVDV in various species of animals. Isolation and identification of the virus and its antigens, antibodies and nucleic acids are among the main methods for virus diagnosis. Although BVDV was detected a while ago, most of the research on the virus is mainly focused on various aspects of the viral infection in some domestic animals such as cattle, sheep, goats and pigs (Passler and Walz, 2010). Recently, several studies reported the prevalence of the BVDV in some of the family camelids (Gao *et al,* 2011, Topliff *et al,* 2009). Some experimental studies were carried out to investigate the possibility of BVDV infection in the new world camels including llamas and alpacas (Wentz *et al,* 2003). This study showed the possibility of BVDV infection under experimental conditions however, low seroprevalence was reported in these animals suggesting that cattle is the main source of BVDV infection in camels (Wentz *et al,* 2003). BVDV infection of the cattle during the duration of their pregnancy results in the development of some important phenomena called persistently infected newborn animal (PI) (Grooms, 2004). These PI animals

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are considered as good virus shedders and contribute high risk for the rest of the herd and must be culled (Hilbe *et al,* 2007).

Isolation of BVDV

Virus isolation remains the gold standard technique for the diagnosis of BVDV infection in various species of animals (Gao *et al,* 2011; Peterhans *et al,* 2010; Topliff *et al,* 2009). BVDV isolation can be done from various clinical samples such as blood including sera and buffy coat, nasal swabs and various tissue specimens (Wang *et al,* 2014). The mononuclear cells in the buffy coat of the infected animals represent the ideal target for virus isolation. Meanwhile, the best tissue specimen for the BVDV isolation is the payer's batches in the regional lymphoid tissues, the mesenteric lymph nodes, spleen and the thymus (Turin *et al,* 2012). Based on the cytopathological changes triggered by BVDV infection in cell culture, two types of the virus were identified; the cytopathic (CP) and the non-cytopathic strains of BVDV (NCP) (Gillespie *et al,* 1962). To distinguish NCP-BVDV that show exaltation of Newcastle disease virus (END)+ from NCP-BVDV-END-, END method and interference with VSV were used, however, these techniques require the use of high virulent strains of NDV and VSV, necessitating the use of high laboratory containment measures. Alternatively, a safer technique termed Competitive Virus Assay (CVA) was developed to titerate either BVDV-END+ or BVDV with another heterologous interference phenomenon (Muhsen *et al,* 2013). One of the main limitations of the BVDV isolation from sera of young calves below 3 months of age is the presence of high titres of neutralising antibodies which inhibit virus replication in cell culture (Zimmer *et al,* 2004).

Detection of BVDV antigens

Antigen Capture -ELISA (AC-ELISA) is one of the most common technique for the detection of BVDV antigens in clinical samples including whole blood, nasal swabs as well as some skin lesions especially skin notch (Grooms, 2004). The fluorescent antibody technique (FAT) can also be used for the detection of BVDV antigens in some BVDV infected tissues (lymph nodes, spleen, etc) (Grooms, 2004). Immunohistochemistry (IHC) is also used to detect the BVDV antigens in some of the infected fixed tissues for a long time (Seong *et al,* 2015). IHC can also be performed on frozen ear notch tissue samples (Bedeković *et al,* 2011). Both the IFT and IHC techniques were recently used in the detection of the BVDV antigens in some of the PI animals (Edmondson *et al,* 2007).

The performance of commercial diagnostic tests used for virus/antigen detection in the German BVDV eradication program was evaluated on four positive and one negative ear notch as well as four positive and one negative serum samples. Testing of these samples with Erns-based AC-ELISA showed a diagnostic accuracy of 100% while testing of these samples with 7 commercial and one in-house real-time RT-PCR (rRT-PCR) revealed high agreement (Wernike and Beer, 2019). A targeted antigen may affect the ability of the ELISA test to detect BVDV antigen. In this regards, Erns-based AC-ELISA rather than NS2/3-based AC-ELISA was able to detect BVDV antigen from all over the skin of PI calves, though both tests were equally able to detect the antigen in the recommended sample, from ear notch (Vander Ley *et al,* 2012). Using commercial Erns-based AC-ELISA, tail skin biopsy and nasal swabs were reported to provide similar detection rates to that obtained with ear notch, while it was 92% for the serum and moderate to very low with conjunctival, oral, rectal, vaginal /preputial swabs from PI animals (VanderLey *et al,* 2011). The presence of a colostrum-derived antibody may interfere with antigen detection in serum, nasal and saliva, but not in-ear notch, samples from PI calves for up to 3 weeks (Lanyon *et al,* 2014c). To avoid such interference, a test can be performed on supernatant obtained from treating serum with ethylenediamine tetra-acetic acid at pH 5 ± 0.5, boiling and centrifugation (Lanyon and Reichel, 2016).

The antigenic similarity of Erns and NS2/3 in BVDV-1, -2 and -HoBi were higher than antigenic similarity in E2 protein. Comparing the performance of commercial Erns-based AC-ELISA in detecting BVDV-1,-2 and -HoBi antigens showed its ability to detect all types at the same sensitivity (Bauermann *et al,* 2012). However, due to variation in BVDV and reliance on commercial diagnostic assays, like AC-ELISA, on one or two monoclonal antibodies, the possibility of false negativity should be kept in mind. Hence, BVDV tests may require regular evaluation (Yan *et al,* 2016b, Gripshover *et al,* 2007).

The AC-ELISA was used to detect BVDV-antigen in pneumonic lungs from an apparently healthy camel in Sudan. Out of 474 and 186 lungs, AC-ELISA detected 28 (9%) and 13 (7%) positive samples, respectively (Saeed *et al,* 2015, Intisar *et al,* 2010). To confirm the results of the AC-ELISA, the 13 BVDV-positive lungs were tested again with FAT and RT-PCR, which generated compatible results (Intisar *et al,* 2010). Similarly, among 214 samples

of abomasal contents collected from camel aborted fetuses in Iran, AC-ELISA detected 27 (12.61%) while RT-PCR detected 32 (14.95%) BVDV-positive samples (Dehkordi, 2011). Combining of two ELISA tests, one to detect antibody and the other to detect antigen, for testing of the same sera panel showed unexpectedly high BVDV-antigen prevalence of 22% in Egypt (El Bahgy *et al,* 2018) and 41.4% in Algeria (Saidi *et al,* 2018) with seroprevalence of an anti-BVDV antibody of 33% and 9% respectively (Table 1).

The development of a rapid, sensitive and specific assay to detect BVDV-antigen under field conditions would be advantageous for control and eradication programs. Immunochromatography with anti-NS3 monoclonal antibody was developed and used to detect BVDV in leukocyte extract with sensitivity and specificity of 100% and 97.2%, respectively, relative to virus isolation (Kameyama *et al,* 2006). Similarly, immunochromatography assay based on recombinant E2 protein and chicken anti-E2 IgY was developed to detect BVDV antigen and showed an agreement of 90.91% with RT-PCR results (Zhang *et al,* 2016). A commercial rapid test based on lateral flow technology was evaluated for detection of BVDV in whole blood, plasma and ear notch from PI calves. Results revealed a sensitivity and specificity of ≥96%, relative to AC-ELISA, in all tested sample types with the ability to detect both BVDV-1 and -2 (Rammelt *et al,* 2013).

Detection of BVDV antibodies in sera and milk

Serological techniques are useful in the diagnosis of viral infections in the case of many viruses. However, due to the ubiquitous nature of the spread of the BVDV among the various population of animals and the massive administration of BVDV vaccines, thus, we have to use various serological techniques with extreme caution to diagnose BVDV infection in animals. Typically, seroconversion is the most feasible approach to use various serological techniques in the diagnosis of BVDV infections in animals. This can be achieved by the collection of paired serum samples, one sample should be collected at the acute stage of the disease while the other sample should be collected during 4 weeks apart from the acute sample and during the convalescent stage of the viral infection (Edmondson *et al,* 2007b). It is highly recommended to screen young animals under 6 months of age for possible BVDV exposure. This is to identify if the virus is circulating in some young animals and before using the potentially infected animals for breeding purposes. There are some serological techniques that can be applied to distinguish between the BVDV-1 and the BVDV-2 (Edmondson *et al,* 2007b), especially those targeting E2 protein (Bauermann *et al,* 2012). Several serological tests have been used to detect anti-BVDV antibodies in serum and to a lesser extent in milk, including Virus neutralisation test (VNT), Enzyme-Linked Immunosorbent assay (ELISA), Agar Gel Immunodiffusion test (AGID), Complement fixation test (CFT), Indirect Immunofluorescence Assay (IFA), Western Blotting and peroxidase linked assay. The two former tests are the most popular assays in diagnostic laboratories (Sandvik, 2005).

The virus neutralisation assay is one of the most common serological techniques used to diagnose

Table 1. Summary of the BVDV prevalence studies in camel in Arabian Peninsula and surrounding countries (with results of seroprevalence).

Country	# and type of samples	Test	Prevalence	Reference
Sudan	260 sera	i-ELISA*	84.60%	(Intisar *et al,* 2010)
	186 pneumonic lungs	AC-ELISA /FAT/RT-PCR	7%	
Iran	214 abomasal contents from aborted fetuses	AC-ELISA	12.61%	(Dehkordi, 2011)
		RT-PCR	14.95%	
Sudan	474 peumonic lungs	AC-ELISA	9%	(Saeed *et al,* 2015)
Egypt	200 sera	c-ELISA	33% (47.5% smuggled, 11.2% local	(El Bahgy *et al,* 2018)
		AC-ELISA	22% (31.6% smuggled, 7.5% local)	
KSA	182 sera	ELISA	29.10%	(Khalafalla *et al,* 2017)
	97 uterine swabs from camel with reproductive failure	RT-PCR	0%	
Algeria	111 sera	c-ELISA	9%	(Saidi *et al,* 2018)
		AC-ELISA	41.40%	

* with anti-camel conjugate

BVDV infection in animals. It is highly sensitive and specific (Sandvik, 2005), hence it remains the gold standard for the detection of antibodies against BVDV (Wernike and Beer, 2019). VNT can be used to detect and distinguish antibodies against different types and subtypes of BVDV (Minami *et al,* 2011). It is highly recommended to assess both the acute and convalescent samples using the same reference strain to avoid any misinterpretation of the obtained results (Edmondson *et al,* 2007b, Grooms, 2004). However, the use of different virus strains and cells lines made it difficult to compare results from various laboratories (Dubovi, 2013). Additionally, VNT is laborious, costly, time-consuming, needs well-trained workers and limited to virology laboratories (Sandvik, 2005). To overcome many of these disadvantages, a recombinant BVDV that expresses EGFP was constructed and used in flow cytometry-based-VNT (FC-VNT). The FC-VNT showed the ability to be automated, high-throughput, reproducible and can be performed in 48 hours (Gebauer *et al,* 2014). Colostrum has a cytotoxic effect on cells, impairing the use of VNT for the detection of anti-BVDV antibodies. A modified VNT (mVNT) was developed to overcome this challenge. In mVNT, a high density of MDBK cells was incubated for 3 hours to prevent detachment of cells from the well surface. The cytotoxic effect of colostrum complement on cells was minimised by heat inactivation of the colostrum and reducing the incubation time of colostrum-virus mixture on cells for 1 hour followed by five times washing of MDBK cells with Dulbecco modified Eagle medium (DMEM). The mVNT showed sensitivity and specificity of 100% compared with standard VNT and showed a high correlation between antibody titers in serum and colostrum (Bedeković *et al,* 2013).

ELISA is a widely used serological technique to diagnose BVDV in several clinical samples including blood and milk samples. The Ig-based ELISA is commonly used to detect the antibodies in sera of infected animals rather than for the detection of antigens (Edmondson *et al,* 2007b, Grooms, 2004). It is mandatory to know the vaccination history of the tested animals to avoid any misinterpretations in case of the vaccinated animals (Lanyon *et al,* 2014b). Positive results for serum samples collected from pregnant cattle indicated previous infection or vaccination history with the BVDV (Lanyon *et al,* 2014b). Negative results of screening a statistically representative sample per each herd using the Ig-based ELISA for BVDV may suggest the PI animals. Further confirmation should be conducted to identify the PI animals.

The performance of several commercial ELISA kits for the detection of anti-BVDV antibodies in sera and milk were evaluated in several laboratories participating in German BVDV eradication program. Results showed that some commercial ELISA kits repeatedly generate false-negative results, especially for milk, suggesting its need for optimisation (Wernike and Beer, 2019). Similar studies in the context of the Belgium BVDV eradication programme showed that using of indirect ELISA (i-ELISA) for testing individual serum samples from unvaccinated animals generate the best results for sero-monitoring (Hanon *et al,* 2017, Hanon *et al,* 2018). Pooling of serum samples from viremic seronegative cattle with seropositive sera did not generate false-negative results in i-ELISA and c-ELISA (Graham *et al,* 2019). Similarly, ELISA-detection of anti-BVDV antibody in serum and individual or bulk milk samples showed good agreement (Lanyon *et al,* 2014a). However, using of commercial ELISAs to detect antibodies in bulk milk showed sensitivity and specificity of 70 to 92% and 77 to 88%, respectively, compared to ELISA detection of antibodies in sera at herd level (Eiras *et al,* 2012). It has been reported that there was a diagnostic gap for up to 12 days after cattle parturition for ELISA-detection of anti-BVDV antibody in serum, but not by VNT. In contrast, colostrum was strongly seropositive in ELISA after parturition, though positivity decreases in the next few days (Bachofen *et al,* 2013).

Unlike VNT, the ELISA test is mostly developed and validated for use in certain species, mostly bovine and linked to species-specific reagents, limiting their use in heterologous host species. Competitive or blocking ELISA (c-ELISA) may not link to species-specific reagents but need to be validated for the targeted animals' species (Dubovi, 2013). A study on the diagnostic performance of two bovine commercial ELISAs and AGID test to detect anti-BDV and anti-BVDV in sheep showed that all tests have a specificity of 100% but varies widely in their sensitivity. The authors recommend optimising the threshold value of the ELISA test to obtain >95% diagnostic sensitivity and specificity (Evans *et al,* 2017). Similar optimisation of bovine diagnostic tests would be necessary before using in camels and other species. A study that involved both ELISA and VNT (table 2) showed that out of 812 samples tested with c-ELISA, three positives and 2 suspected samples turned to be negative when tested with VNT. The author concludes that the used ELISA test provides reliable results in the detection of anti-BVDV in camel sera (Taha, 2007).

Several investigations were performed to determine the seroprevalence of BVDV in camels in Arabian Peninsula and surrounding countries. Different serological tests were used, with most studies before 2010 using the VNT while most studies performed thereafter were based on the ELISA test (table 2). Seroprevalence of BVDV in camel varies widely with the majority of the investigations reported it between 5% and 30%. However, a comprehensive study based on a random representative sample that involves the whole camel population seems scarce. Seropositivity of 9% and 29.1% were reported respectively from Algeria and Saudi Arabia randomly collected serum samples, (Khalafalla *et al,* 2017; Saidi *et al,* 2018). Seroprevalence as high as 52.5% and 58.7% were also reported at subnational administrative divisions in Egypt (Zaghawa, 1998) and Turkey (Erol *et al,* 2020), respectively. The highest seroprevalence of 84.6% was reported from a Sudanese national study based on randomly collected samples (Intisar *et al,* 2010). Noteworthy, this seems to be the only study that performed with species-specific anti-camel conjugate in i-ELISA. Most of the studies in this regard were performed with VNT or c-ELISA format; while a couple of studies used i-ELISA format but without specifying the used conjugate or with other than anti-camel conjugate. As stated earlier, evaluation of several commercial ELISA assays showed that, for serum, i-ELISA targeting the whole virus was more sensitive than i-ELISA targeting NS3 or other competitive ELISA (c-ELISA) (Hanon *et al,* 2017).

Detection of BVDV-Nucleic acids

Nowadays, detection of the viral nucleic acids in clinical specimens is the gold standard technique for BVDV diagnosis. Some molecular diagnostic assays, especially the RT-PCR have been used successfully used for the detection of various BVDV strains (Laamanen *et al,* 1997). Some studies reported that the stability of the BVDV-1-RNA in the foetal brain was much higher than that in foetal skin, muscle, ear, or pooled tissues (Ridpath *et al,* 2014). The copy number of viral RNA detected in peripheral blood mononuclear cells (PBMCs), ear notches and the hair bulbs were much higher than that detected in plasma or dry blood (Zoccola *et al,* 2017). The detectability of BVDV in buffy coat samples was significantly higher than that in serum or nasal swab samples from transiently infected calves (Peddireddi *et al,* 2018). Faecal and aerosol samples from persistently infected cattle are suitable samples for the detection of

Table 2. Seroprevalence of anti-BVDV antibody in camel in Arabian Peninsula and surrounding countries.

Country	# of serum sample	Test	Result	Refer
Oman	30	SNT	6.7%	(Hedger *et al*, 1980)
Sudan	102	SNT	15.70%	(Bornstein and Musa, 1987)
UAE	1000		9.2% for breeding females 3.6% for racing females	(Wernery and Wernery, 1990)
Egypt	59	SNT	52.5%	(Zaghawa, 1998)
Egypt	365	SNT	1% for abattoir group, 1.7% for camel in contact with cattle	(Eisa, 1998)
KSA	2472		18%	(Al-Afaleq *et al*, 2007)
UAE	812	c-ELISA, SNT	0%	(Taha, 2007)
UAE	1119		1.6%	(Wernery *et al,* 2008)
Iran	137	SNT	19.70%	(Raoofi *et al,* 2010)
Sudan	260	i-ELISA*	84.60%	(Intisar *et al,* 2010)
Egypt	165	SNT	14.54%	(Elbayoumy *et al,* 2013)
Iraq	88	i-ELISA	13.63%	(Al-Rubayie, 2016)
Egypt	200	c-ELISA	33% (47.5% for smuggled from sudan, 11.2% local camel)	(El Bahgy *et al,* 2018)
KSA	182	ELISA	29.10%	(Khalafalla *et al,* 2017)
Algeria	111	c-ELISA	9%	(Saidi *et al,* 2018)
Egypt	92	i-ELISA	27.20%	(Malek and Madkour, 2017)
KSA	316	c-ELISA	10.8%	(Intisar, 2019)
Turkey	92	SNT	58.70%	(Erol *et al,* 2020)

* anti-camel conjugate was used.

BVDV-1 by most common molecular techniques such as the TaqMan real-time RT-PCR (rRT-PCR) or SYBR Green rRT-PCR, respectively (Liang *et al*, 2019; Hou *et al*, 2020). The use of tissue swabs for rRT-PCR detection of BVDV was reported to yield comparable results to that obtained from tissue pieces with the possibility of reducing lysis time (Errington *et al,* 2014). Like blood samples, hair samples (30-100 pooled hairs from each animal) were successfully used to detect PI calves using rRT-PCR (Singh *et al,* 2011). With pooled samples of ear notch or sera, the Ct of the rRT-PCR significantly correlated with pool size (number of animals). The cost-effective pool size to screen with rRT-PCR was reported to be 25 or 50 samples at BVDV prevalence of 0.75-2% and 0.25 to 0.5%, respectively (Yan *et al,* 2011). Both the proper sampling, the presence of the pre-existing anti-BVDV antibodies in sera of tested animals affects the performance of serological techniques. For example, the presence of a higher level of anti-BVDV neutralising antibody reduces the number of BVDV positive RT-PCR days and increases the average Ct value over-tested days for buffy coat samples (Peddireddi *et al*, 2018). Colostrum-derived IgG, on the other hand, showed no inhibitory effect on the performance of the RT-PCR used to detect BVDV in calves blood at the age of 48 hours (Chigerwe and Crossley, 2013). Direct amplification of the BVDV-1- 5'UTR from as low as 0.5 µl of the heat-treated serum or plasma samples with no RNA extraction was successful in producing sufficient PCR product for sequencing purposes (Bachofen *et al*, 2013b). Similarly, direct detection of BVDV in hair bulbs-supernatant was capable of detecting all PI animals using TaqMan rRT-PCR (Zoccola *et al*, 2017). Magnetic particle technology was also used successfully to shorten the time of extraction of BVDV from blood or serum samples (Aebischer *et al*, 2014a). A chemically treated paper that upon adding biological samples serve to lyse cells and stabilise nucleic acids was developed and termed Fast Technology Analysis (FAT) card. Using this card for collection and transport of nucleic acid of BVDV and other bovine respiratory viruses was reported to maintain nucleic acid in a quality suitable for rRT-PCR testing over a wide range of temperature (-14°C to 47°C) for 14 days (Liang *et al,* 2014).

There are two new types of amplification assays (1) the thermal cycling techniques with the ligase chain reaction (LCR) and (2) the isothermal amplification techniques like loop-mediated isothermal amplification (LAMP), Rolling circle amplification (RCA) and hybridisation chain reaction (HCR) (Monjezi *et al*, 2016)

The development of an RT-PCR capable of detecting all types the BVDV was a fundamental issue for virus monitoring and surveillance programs. The genetic diversity of Hobi-like viruses was reported to represent a challenge for the developing diagnostic RT-PCR and rRT-PCR assays for this virus (Moorthy *et al*, 2019). Additionally, frequent mutations within the 5'UTR region reduce the performance of the regular rRT-PCR (Yan *et al,* 2016a). The conventional RT-PCR (cRT-PCR) (Monteiro *et al*, 2019), TaqMan-rRT-PCR (Losurdo *et al*, 2015) and the Multiplex-TaqMan-rRT- PCR (Mari *et al*, 2016) capable of detecting all three BVDV types have been reported with a detection limit of as low as 100 to 101 viral RNA copies in case of the TaqMa- rRT-PCR. However, failure in the detection of BVDV or HoBi-like viruses is not uncommon. For example, cRT-PCR and rRT-PCR designed for detection of either BVDV or HoBi-like viruses were used in the detection of HoBi-like virus in serum, buffy coat and ear notch from PI calves. According to the used test and sample type, the percentage of false-negative was 17-75% using BVDV cRT-PCR, 0-17% using BVDV-rRT-PCR, 13-25% using HoBi-like cRT-PCR and 4-38% using HoBi-like rRT-PCR. (Bauermann *et al*, 2014). Expanding the diagnostic capacity to include other viruses related to bovine Pestiviruses was also attempted. The cRT-PCR followed by ELISA was used to detect the PCR product successfully used to simultaneously detect BVDV-1, BVDV-2, as well as the Border disease virus in cattle, sheep and goats with high sensitivity, specificity and detection limit of 10 TCID50/ml (Dubey *et al*, 2015). Similarly, triplex TaqMan-rRT-PCR was successfully used to detect and differentiate wild-type classical swine fever virus, hog cholera lapinised vaccine (HCLV) and BVDV-1 with a detection limit of 3.2 TCID50 for BVDV-1 (Zhang *et al*, 2012). Some studies developed a five-plex cRT-PCR was developed to detect five viruses related to bovine respiratory disease (BRD) or bovine enteric disease (BED), followed by differentiation on an electronic microarray cartridge (Thanthrige-Don *et al*, 2018).

Another multiplex cRT-PCR assay was developed to do simultaneous detection of three BED viruses, including BVDV, with dual-priming oligonucleotide (DPO) primers combined with nanoparticles assisted PCR (DPO-nano-PCR). Results showed that the DPO-nano-PCR was able to detect the targeted pathogens with higher specificity and sensitivity compared to the cRT-PCR (4.09X101 versus 4.09X104 copies/µl regarding BVDV) (Wang *et al*, 2019). Similarly, another multiplex rRT-PCR was

developed to simultaneously screen 16 pathogens involved in the BRD syndrome, including the BVDV (Kishimoto *et al*, 2017). Similarly, it was used to detect and differentiate between several notifiable diseases of cattle, including BVDV (Wernike *et al*, 2015), with a detection limit of ≤100 copies/reaction.

Several isothermal amplification techniques were developed for the detection of BVDVs. The reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) was adapted to detect BVDV-RNA with high specificity and sensitivity. This technique was used successfully to detect as low as 4.67X100 RNA copies of the viral genome (Fan *et al*, 2012) and 70 PFU/ml of the virus (Tajbakhsh *et al*, 2017). A single-step RT-LAMP test for detection and differentiation of BVDV-1-2 was described with a detection limit of 103 copies of synthesised DNAs (Mungthong *et al,* 2021). For the rapid detection of BVDV, an assay based on recombinase polymerase amplification (RAP) using primers and probes targeting the 5'UTR was developed. After 15 minutes of amplification at 38°C, the RAP product can be detected on the lateral flow dipstick (LFD). RAP-LFD assay was specific with a detection limit comparable to rRT-PCR (20 copies/reaction) (Hou *et al*, 2018).

Three promising techniques, including LAMP, recombinase polymerase amplification (RPA) and high-speed rRT-PCR (hrRT-PCR), were tested for suitability for field use and performance compared with standard rRT-PCR for detection of BVDV. All the three assays may provide results within 30 minutes, hrRT-PCR was superior regarding sensitivity and speed of assay design, the LAMP was the best assay regarding specificity, simplicity (portable equipment) and cost-effectiveness, while RPA was as simple as LAMP (Aebischer *et al*, 2014b). The cross-priming amplification (CPA) assay targeting the 5'-UTR region of the BVDV was successfully used in detecting BVDV-1 and -2 with a detection limit of 3500 and 80000 copies/reaction, respectively, in samples from PI animals (Kuta *et al*, 2015). The other isothermal technique is the hybridisation chain reaction (HCR) assay with colorimetric detection of gold nanoparticles. HCR assay based on a salt-induced aggregation of unmodified gold nanoparticles was developed to detect BVDV-RNA with a detection limit of 0.1 and 0.008 TCID50/ reaction for visual and spectrophotometer measurement, respectively (Monjezi *et al*, 2016). Analogous detection of unamplified BVDV-RNA was successful with a detection limit of 200 TCID50/ ml by naked eye (Heidari *et al*, 2016). Similar HCR assay based on peptide nucleic acid (PNA) induced aggregation of unmodified gold nanoparticles showed high sensitivity with a detection limit of 10.48 and 1.05 ng/reaction of BVDV-RNA for visual and spectrophotometer measurement, respectively (Askaravi *et al*, 2017).

An assay with dual ssDNA aptamers capable of binding to BVDV-1, one for immobilisation of BVDV-1 (capturing probe) and the other conjugated with gold nanoparticles (reporting probe), was developed and successfully used to detect as low as 800 copies per ml, a sensitivity equivalent to that of rRT-PCR (Park *et al*, 2014). Another rapid screening test is a dot blot assay that developed based on the conjugation of BVDV specific affinity peptides with unmodified gold nanoparticles. Conjugate is added to bind to immobilised BVDV on nitrocellulose strips; then copper nano-polyhedral shells are added to amplify a signal. The detection limit of this test was reported to be 4.4 copies/ml (Kim *et al*, 2020). For *in situ* detection and quantification of BVDV in single-cell, RNA probe was used to specifically bind Npro-Erns coding region in BVDV-2a followed by flow cytometry and fluorescent microscopy (Falkenberg *et al*, 2017). Next-generation sequencing was successfully used to sequence a panel of 21 BVDV isolates and produce near-full complete genome sequences (Neill *et al,* 2014).

Future perspectives on the diagnosis of BVDV in Camelids

Despite the ubiquities nature of the spread of BVDV among cattle and other species of animals, still is still known about its prevalence, pathogenesis and diagnosis in dromedary camels. Currently, well-known diagnostic assays and approaches are adopted for the diagnosis of BVDV in other species of animals including dromedary camels. It is highly recommended to develop specific diagnostic assays for the detection of the BVDV in dromedary camels.

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MICROSCOPIC, SEROLOGICAL AND MOLECULAR SCREENING OF *Theileria annulata* IN CAMELS (*Camelus dromedarius*) OF SAUDI ARABIA

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ABSTRACT

The present study was aimed to evaluate the prevalence rate of *Theileria* spp., especially *T. annulata*, in camels of Taif Governorate, Saudi Arabia. A total of 102 blood samples were collected from camels. Parasitological examination of buffy coat smear, ELISA-IgG, and PCR (targeting 16S rRNA and cytochrome b gene sequences) assays were done for *T. annulata* infection detection. The results report a high prevalence rate of *T. annulata* in the examined dromedary's population using ELISA-IgG (32.4%), followed by targeted PCR (25.5%). Then lower detection was reported by buffy coat Giemsa stained (10.8%). Statistical analysis using one-way ANOVA followed by Tukey's at $P < 0.001$ showed significant variation in values within different diagnostic tools and age categories. Both females and males were at risk of parasitic infections, but females have a higher incidence (17.6%, 15.7%, 6.8%). Young age (X≤ 3 years) camels were the most infected category (50.0%, 44.4%, 22.2%) according to age from elder ones. These results could be a baseline for easing a later large-scale epidemiological screening on tropical theileriosis in the camel population in Saudi Arabia.

Key words: *Camelus dromedarius*, KSA, prevalence, Taif, *Theileria annulata*

Theileriosis is one of the most critical tick-borne diseases (TBD) of animals caused by different species of *Theileria*. *Theileria annulata* (*T. annulata*) and *T. parva* are the dominant *Theileria* species that are linked to economic loss and mortality worldwide (Roy *et al,* 2021). In Saudi Arabia, *T. annulata* is the primary causative agent for theileriosis that is transmitted by *Hyalomma* spp. (Hussein *et al,* 1991; De Kok *et al,* 1993). Several studies have reported *T. annulata* from cattle in the eastern province, and Qassim region in Saudi Arabia based on microscopic examination of blood films collected from infected animals (Al-Atiya *et al,* 1991; Hussein *et al,* 1991; Omer *et al,* 2003). However, few studies were done related to dromedary theileriosis in last two decades (Abou-El-Naga *et al,* 2005; El-Fayoumy *et al,* 2005; Hamed *et al,* 2011; Ismael *et al,* 2014; Youssef *et al,* 2015).

The present study aims to assess the prevalence rate of *Theileria* spp., especially *Theileria annulata*, in Taif Governorate, Makkah province, KSA using different diagnostic assays: parasitological, serological, and molecular evaluations in dromedary camels.

Materials and Methods

Three different areas in Taif region were selected from December 2020 to February 2021 to collect blood samples from 102 apparently healthy camels (36 males, aged 1-7 years and 66 females, aged 3-12 years). Blood samples were collected from ear veins (2ml in EDTA tubes) by veterinarians as per their routine examination.

Parasitological diagnosis

Blood and buffy coat smears were done for all samples. All slides were stained by 10% Giemsa, and then examined under a light microscope for *Theileria* spp. diagnosis (Schalm, 1971).

Serological test

According to the instruction manual of Sunlong Biotech® Company (China) blood samples were centrifuged (20 min, 2500 rpm), and then sera were collected for *Theileria* antibodies detection using sandwich-ELISA. Briefly, 50µl of sera in the dilution buffer (with a ratio of 1:4) were added to micro-ELISA strip plate wells (pre-coated with an antigen-specific to *Theileria* spp.). ELISA plate was gently shaken, incubated (30 min, 37°C), and then washed five times with the washing buffer. Next, the reagent of Horseradish Peroxidase (HRP)-conjugate was added to each well, incubated, and then washed. Finally, colour was developed by the addition of chromogen solutions and incubation (15 min, 37°C). Next, 50µl

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of the stop solution was added to terminate the reaction, and then ELISA plate was read at 450nm. Negative and positive controls supplied within the kit were added in separate wells for data calculations. The presence of *Theileria* spp. was determined by comparing data with their cut-off values. Cut-off (critical value) was calculated as the average value of negative control + 0.15, i.e., a negative OD value < cut-off while a positive OD value ≥ cut-off.

DNA extraction by salting out

Low and high salt buffers, TKM1 and TKM2, were used for DNA extraction. Briefly, the same volumes of blood sample and TKM1 (100mM Tris-HC1, pH 7.4, 250mM sucrose, 10mM EDTA) were mixed and incubated at room temperature for 30 min. Then, the mixture was centrifuged (10 min at 4000 rpm). The supernatant was discarded, and then TKM2 (Tris HCl 10 mM pH 7.6, 10 mM KCl, 10 mM MgCl2, 0.4 M NaCl, and 2mM EDTA), SDS (10%), and proteinase k enzyme (10 mg/ml) were added to the pellet and incubated (30 min, 55°C). Protein was removed with the aid of 6M NaCl, and centrifugation. DNA was precipitated in the supernatant by cold ethanol addition. Finally, the DNA pellet was collected by centrifugation (10,000 rpm, 10 mins), dried, and dissolved in 100 µl autoclaved Milli-Q water (Ahmad *et al,* 2007).

PCR amplification

Firstly, primers were used to target the small subunit ribosomal 16S rRNA sequence of *Theileria* spp. (TH989: 5′-AGTTTCTGACCTATCAG-3′ and TH990: 5′-TTGCCTTAAACTTCCTTG-3′). Then positive *Theileria* spp. PCR samples were subjected to another PCR that targeted cytochrome b gene sequence specifically for *T. annulata* (using forward TCyt1F: 5′- ACTTTGGCCGTAATGTTAAAC-3′ and reverse TCyt1R: 5′- CTCTGGACCAACTGTTTGG-3′ primers) (Masiga *et al,* 1992; Figueroa *et al,* 1993). For both reactions: PCR amplification was done in a total reaction volume of 20 µl: 7 µl H2O, 1 µl (20 pmole) of each forward and reverse primers, 1 µl extracted DNA, and finally 10µl of 2× master mix. The PCR reaction was set up with initial denaturation at 95°C (5 mins), 40 cycles of denaturation at 94°C (30 s), primer annealing at 55°C (60 s), and then primer extension at 72°C (60 s). The final extension at 72°C (10 mins) was necessary done for complete amplification. PCR products of all samples were separated on 1.5 % agarose gel for about 60 min (at 100V), visualised, and then photographed by a gel documentation system. PCR products' size was visually determined by comparing them with a known low molecular weight marker (50-1500 bp).

Statistical analysis

All data collected from parasitological, serological, molecular were analysed statistically. Student t-test was used to differentiate between two related groups. One-way ANOVA was conducted to differentiate between different groups, followed by Tukey's multiple comparisons test using GraphPad software (GraphPad® 2017, San Diego, CA, USA). *** indicates $P \leq 0.001$, ** indicates $P \leq 0.01$, * indicates $P \leq 0.05$ and ns (non-significant) means $P > 0.05$.

Results

Buffy coat examination

Parasitological diagnosis in the whole blood smear showed negative results of all the studied samples (n=102). However, examination of buffy coat smear using Giemsa stain showed n=11 (10.8%) positive *Theileria* spp. (Table 1). *Theileria*'s schizont nuclei appeared stained with Giemsa in the cytoplasm of the infected cells, as shown in Fig 1. According to gender: female positive samples 7/102 (6.8%) showed a statistically high significance in comparison to male samples 4/102 (3.9%) according to the total number of samples at $P < 0.01$ by using student t-test. According to age, young camels from (X≤ 3 years) show the highest prevalence (22.2%), followed by 3>X≤ 6 years (13.9%), then 6>X≤9 years (6.5%), but old camels with age ranges from 9>X≤12 years doesn't show any infection according to parasitological evaluation (0%) ($F (3, 8) = 8183$; $P < 0.001$; R square = 0.9997) by using one-way ANOVA. Tukey's test reports a highly significant difference between all diagnostic groups at $P < 0.001$.

Seroprevalence assessment

Of the 102 sera, samples were diagnosed serologically for the presence of *Theileria* spp. IgG, n=33 (32.4%) positive samples were reported. Table 1 shows a highly significant difference between total positive samples by using different diagnostic assays. In which, IgG seroprevalence using ELISA shows the highest significance (32.4%), followed by targeted PCR (25.5%), and then lower detection was reported by buffy coat Giemsa stained (10.8%) at $F (2, 6) = 8937$; $P < 0.001$; R square = 0.9997 by using one-way ANOVA followed by Tukey's test reports at $P < 0.001$.

Table 1 shows a high significance increase of *Theileria*'s seroprevalence detection in female camel samples 18/102 (17.6%) than males 15/102 (14.7%)

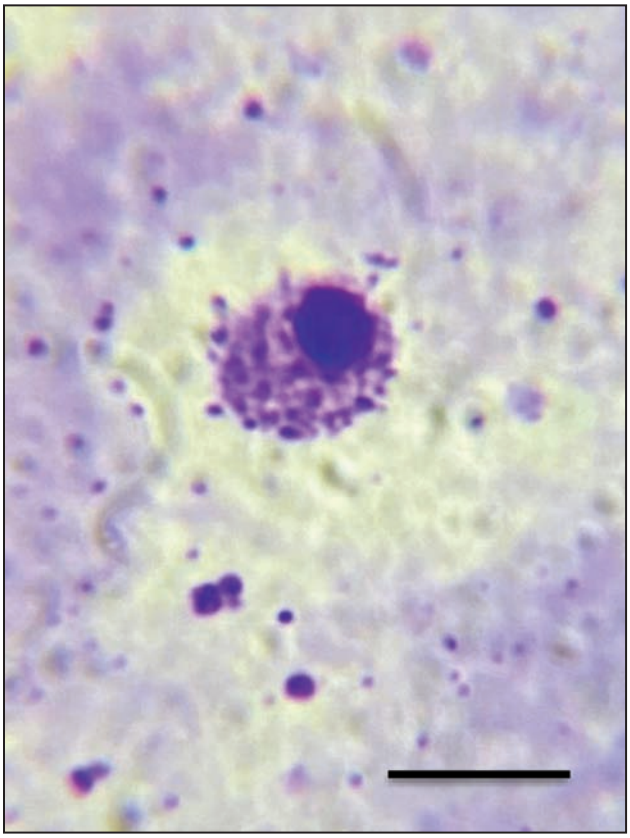

Fig 1. Light micrograph of camels' blood buffy coat smear showing Giemsa-stained schizont nuclei of *Theileria* spp. in the cytoplasm of an infected erythrocyte. Scale-bars: 10 µm.

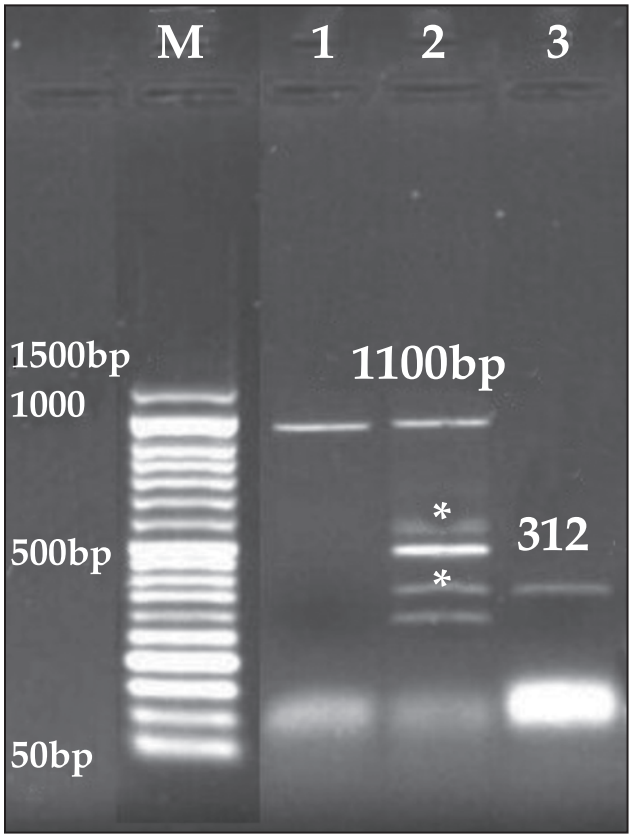

Fig 2. Representative photograph of agarose gel stained with ethidium bromide (1.5%) showing PCR product of small subunit ribosomal 16S rRNA sequence of *Theileria* spp. at 1100 bp (lanes 1 and 2) and cytochrome b gene sequence of *Theileria annulata* at 312 bp (lane 3). Lower bands in lane 2 are non-specific bands. Lane M: Low molecular weight marker (50-1500 bp).

at P ≤ 0.001 using the student t-test. According to age: young camel samples (X≤3 years) show high prevalence (50.0%), followed by 9>X≤12 years (37.5%), then 3>X≤ 6 years (30.6%), and finally 6>X≤9 years (22.6%) were the least detected infected samples using ELISA IgG by using one-way ANOVA (F (3, 8) = 1461; P < 0.001; R square = 0.9982) followed by Tukey's test reports at P < 0.001.

Molecular evaluation

Fig 2 shows a successful amplification of the small subunit ribosomal 16S rRNA sequence of *Theileria* spp. at molecular size 1100bp, and cytochrome b gene sequence specific for *T. annulata*

Table 1. Combined results showing gender, number and age of camels, in addition to parasitological, serological and PCR positive results for *Theileria* spp. diagnosis in all studied samples.

	Number (*n*)	Parasitological evaluation (%)	ELISA- IgG (%)	PCR (%)
Gender				
Female	67	7/67 (10.4%), 7/102 (6.8%)**	18/67 (26.9%), 18/102 (17.6%)***	16/67 (23.9%), 16/102 (15.7%)***
Male	35	4/35 (11.4%), 4/102 (3.9%)	15/35 (42.9%), 15/102 (14.7%)	10/35 (28.6%), 10/102 (9.8%)
Age (X, year)				
X≤ 3	18	4/18 (22.2%)^{a}	9/18 (50.0%)^{a}	8/18 (44.4%)^{a}
3>X≤ 6	36	5/36 (13.9%)^{b}	11/36 (30.6%)^{b}	11/36 (30.6%)^{b}
6>X≤9	31	2/31 (6.5%)^{c}	7/31 (22.6%)^{c}	4/31 (12.9%)^{c}
9>X≤12	16	0 (0.0%)^{d}	6/16 (37.5%)^{d}	3/16 (18.8%)^{d}
Total	**102**	**11/102 (10.8%)**	**33/102 (32.4%)**	**26/102 (25.5%)**

Different letters within the same column refer to statistically significant difference between those groups at P ≤ 0.001 using one-way ANOVA followed by Tukey's test. *** indicates P ≤ 0.001, ** indicates P ≤ 0.01, * indicates P ≤ 0.05 and ns (non-significant) means P > 0.05.

at 312 bp in comparison to the low molecular weight marker ranges from 50 to1500 bp. Table 1 indicates a high significance prevalence rate of female camel samples 16/102 (15.7 %) in comparison to male samples 10/102 (9.8 %) at $P \leq 0.001$ using the student t-test. According to age, also samples from young camels ($X \leq 3$ years) show the highest incidence by using PCR technique (44.4%), followed by $3 > X \leq 6$ years (30.6%), then $9 > X \leq 12$ years (18.75%), and the least was referred to $6 > X \leq 9$ years (12.9%) by using one-way ANOVA ($F(3, 8) = 19069$; $P < 0.001$; R square = 0.9999). Tukey's test revealed a highly significant difference between all diagnostic groups at $P < 0.001$.

Discussion

Theileria spp. belongs to the order Piroplasmida is a common pathogen that is transmitted by ticks. Theileriosis is the second most serious hemoprotozoan disease that affects dromedary camels in tropical and subtropical countries, including Saudi Arabia (Al-Khalifa *et al,* 2009; Durrani *et al,* 2012; El Imam *et al,* 2016). The present study reports a high prevalence rate of *Theileria* spp., especially *T. annulata*, in dromedaries by using different diagnostic examinations; ELISA-IgG (32.4%), PCR (25.5%), and microscopic (10.8%) assessment. There are a lot of research studies that report the prevalence of piroplasm genus, including *Theileria* spp., in cattle in Saudi Arabia, but there is a scarcity regarding camels (Ghafar and Amer, 2019). The present results were consistent with Ismael *et al* (2014) who reported theileriosis (38.73%) in dromedaries in Riyadh region, Saudi Arabia according to parasitological examinations of blood samples using Giemsa-stained blood smears.

Microscopically, *Theileria* spp. was detected in Giemsa-stained buffy coat blood smears with a prevalence rate of 10.8% in camels of the present study. Microscopic examination of buffy coat smear is more specific and sensitive than the whole blood for parasitic diagnosis (Chagas *et al,* 2020). However, it is difficult to identify its species due to parasite developmental changes in size and the form in the infected erythrocyte that differs from host to other (Homer *et al,* 2000). Therefore, we have used a PCR to target the 16S rRNA sequence of *Theileria* spp. and cytochrome b gene sequence, specifically of *T. annulata*. Detection of positive samples was increased by using a molecular assay (25.5%) that is more specific, sensitive, and accurate than microscopic examination (10.8%) (Ullah *et al,* 2021). There is a doubt for the exact speciation of *Theileria* protozoa, therefore, recent studies targeting 18S rRNA gene by traditional PCR was recommended for the confirmation of the camel's *Theileria* species (A'aiz *et al,* 2021).

It is not surprising that the prevalence rate was higher by using ELISA-IgG of *Theileria* spp. (32.4%) than targeted PCR (25.5%). ELISA was able to detect past and recent infections, while PCR could screen only the present parasite infection (Reithinger *et al,* 2003). The present study shows a statistically increase of *T. annulata* incidence in females than in male camels using different diagnostic techniques. This could be attributed to stress on females during gestation and milk production, making them more susceptible to Piroplasmids' infection (Barghash, 2010; Zayed *et al,* 2010).

Moreover, the number of females in the present study is more than males, giving a chance to spread infection within females. Furthermore, we have detected *Theileria* infection in all ages, especially young, with different prevalence rates using various diagnostic tools. According to gender, our findings were consistent with previous study El-Naga and Barghash (2016) they have investigated camels in the Northern West Coast of Egypt infected with *Theileria* spp. with the incidence of infection in females (73.3%) higher than males (71.4%), and the maximum rate of infection was found in age range 6-12. The feeding method could have a role in the incidence of disease throughout different ages; all camels were freely grazed in the studied area (Ullah *et al,* 2021). The higher prevalence in young camels could be due to their not fully developed immune system to combat *T. annulata* infection (Ahmed *et al,* 2008) or more caring of the owner with the adult ones. In addition, a lower *T. annulata* prevalence rate in older camels may be due to the development of concomitant immunity due to their recurrent infections throughout their lifetime (Ilhan *et al,* 1998; Gharbi and Darghouth, 2014).

The high prevalence rate of theileriosis in dromedaries in the studied areas could be corroborated to the high prevalence rate of their vectors. ALanazi *et al* (2018) have detected five different tick species on camels from Saudi Arabia with various prevalence: *Hyalomma dromedarii* (70.6%), along with *Hyalomma impeltatum* (25.3%) *Hyalomma anatolicum* and *Rhipicephalus turanicus* were less prevalent (<2%), and *Haemaphysalis* spp. ticks were found on camels in few limited provinces. They have reported that *H. dromedarii* tick was the only tick from those five species that are positive for *Theileria* spp. DNA.

Therefore, *H. dromedarii* tick acts as a vector of *Theileria* spp. in camels of Saudi Arabia. Their findings agreed with other studies, in which they have identified *H. dromedarii* and *H. anatolicum* as main ticks for *Theileria annulata* transmission in cattle and camel in Hofuf, the Eastern Saudi Arabia region (Omer *et al,* 2021) and *H. dromedarii* in the United Arab Emirates (Al-Deeb *et al,* 2015).

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Declarations

Ethics approval and consent to participate

Data and samples were collected from veterinarians as per their periodic examination according to the ministerial recommendation. Ministry of Environment, Water and Agriculture (KSA) permitted to collect cattle samples from different locations in Taif for our research study with the number 106082/1074/1442, date 21-02-1442H. Sample's collection and all experimental procedures were performed following a national ethical requirement (National Committee of Bioethics (NCBE) at King Abdulaziz City for Science and Technology (KACST) with number: 10023117, valid till 01 October 2023).

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Authors' Contributions

Both authors participated in the design of the study, carried out molecular evaluation and approved the final manuscript. Jamila S. Al Malki collected samples and performed parasitological assay. Nahed Ahmed Hussien performed data analysis and wrote the article draft.

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MORPHOMETRIC STUDY ON THE GOBI RED BULL BACTRIAN CAMEL

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ABSTRACT

The experimental data comes from randomly selected 40 bull camels in the 2020 Gobi Red Bull Camel Competition organised in North Urad Banner. In this study, 21 kinds of the actual body size parameters were measured in each camel with an average age of 9.28 year and the main statistical body size of bull camels include: body height 176.30 cm, body length 151.40cm, chest circumference 240.03 cm and ankle circumference 23.68 cm, respectively. At the same time, the front view and side view images of bull camel were collected by digital camera, and then the photo data of body size were obtained with Photoshop. The corresponding photo data of body height is 61.52 cm, body length is 52.13 cm, and line chest circumference is 20.48 cm, respectively. Based on on-site measurement scale λ=2.87, the actual body data of bull camel were obtained from the corresponding photo data with relatively small error. The three-dimensional chest circumference of bull camel was 4λ times as the line chest circumference on bull camel photo. Many related golden ratio φ were found in Bactrian camel body size data ratio. An optimised formula for estimating the weight of bull camel based on photo data LI_{PD} were derived: $Y(kg)=-773.57+(2.66+4.06\beta_{ABD})\ \lambda\ LI_{PD}$ (cm).

Key words: Bactrian camel, golden ratio, morphometry, non-contact measurement, photo data

The Gobi Red Bactrian camel is mainly distributed in the Gobi grassland of north western China and southern Mongolia. The camel wool is purple red, so it is called "Gobi Red Camel" (Batu Suhe, 2019). Like other Bactrian camels the Gobi Red Camel is capable of survival in harsh living environments, being able to consume diets that are often toxic to other mammals and can tolerate extreme water and food deprivation (Surong *et al*, 2018; Dongjirzhab, 2019). It not only provides meat, milk, wool and other living materials, but also is used for riding and transportation. The anatomy of Bactrian camels is scarcely studied (Surong and Dugarsiren, 2013). Present study was, therefore undertaken to study the morphometriy of the Gobi red bull Bactrian camel.

Materials and Methods

At present study, 40 bull camels were randomly selected from Gobi Red Bull Camel Competition organised in North Urad Banner in the winter of 2020, and their body height, body length, chest circumference, ankle circumference and other actual body sizes were measured. Meanwhile, the corresponding size data was extracted from the photos of each bull camel. Through comparing the actual body data and photo data of bull camel, the error between the actual body data and estimated data from photo data was determined. Finally, various ratio analyses were carried out on the body size data of bull camels.

Measurements and analysis

The definition of height, length and width in the body size of Bull camel

In present study, 21 body size parameter of Gobi red bull camel were defined based on camel anatomy (Surong *et al*, 2013; Su xuebin, 1983), as shown by solid line in Fig 1. The abbreviations of diverse body size parameters are given below but are not repeated in all the tables.

Height of the body (HB): the vertical distance from the bottom of the hump to the ground.

Height of the shoulder (HS): the vertical distance from the highest point of the scapula to the ground.

Height of the pars sacralis (HP): the vertical distance from the highest point of the pars sacralis to the ground.

Height of the fore leg (HFL): the vertical distance from the root of the front thigh to the ground.

Height of the back leg (HBL): the vertical distance from the root of the back thigh to the ground.

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Height of the fore knee (HFK): the vertical distance from the front knee to the ground.

Height of the back knee (HBK): the vertical distance from the back knee to the ground.

Height of the first hump (HFH): the vertical distance from the top of the first hump to the bottom.

Height of the second hump (HSH): the vertical distance from the top of the second hump to the bottom.

Length of the body (LI): the incline distance from the shoulder to the hip.

Length between two humps (LBH): the horizontal distance from the back edge of the front hump to the front edge of the second hump.

Length of the first hump (LFH): the horizontal distance from the front edge of the first hump to the back edge.

Length of the second hump (LSH): the horizontal distance from the front edge of the second hump to the back edge.

In addition, based on camel anatomy (Surong *et al*, 2013; Su xuebin, 1983), the definition of the width and circumference of the bull camel is as follows (not marked on the Fig 1).

Chest circumference (CC): three-dimensional chest circumference formed from the back edge of the front peak down through the centre of the chest.

Line Chest circumference (LCC): the vertical distance formed from the back edge of the front peak down to the base of foreleg in the bull camel photo.

Ankle circumference (AC): The horizontal circumference of the upper third of the forelimb.

Widths of the shoulder (WS): the horizontal distance between the left and right shoulder.

Shoulder width: the horizontal distance between the left and right scapula.

Widths of the pars sacralis (WP): the horizontal distance between the outer sides of the pars sacralis.

Widths of the fore palm (WFP): the distance from the left edge to the right edge of the fore palm.

Length of the fore palm (LFP): the distance from the toe to the heel of the fore palm.

Widths of the hind palm (WHP): the distance from the left edge to the right edge of the hind palm.

Length of the hind palm (LHP): the distance from the toes to the heel of the hind palm.

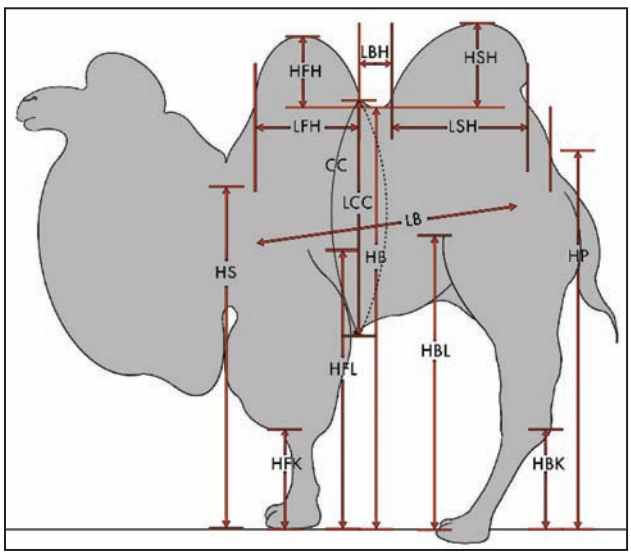

Fig 1. Definition of Bactrian camel body size parameters.

Table 1. The statistical average (AVG) and the standard deviation (SD) of the actual body data of Gobi red bull camel.

item	age	HB	HS	HP	HFL	HBL	HFK	HBK	HFH	HSH	LI
AVG	9. 28	176.30	127.48	135.13	106.33	106.48	46.88	51.73	35.53	33.03	151.40
SD	2.66	5.70	6.30	6.92	6.65	8.69	4.05	5.37	4.49	5.88	11.08
item	LBH	LFH	LSH	CC	AC	WS	WP	WFP	LFP	WHP	LHP
AVG	23.68	43.35	53.18	240.03	23.68	60.73	55.63	21.80	21.80	18.40	19.58
SD	5.48	4.62	8.94	10.51	3.16	5.55	5.95	1.93	1.55	3.24	1.36

Table 2. The result of statistical analysis of photo data and ratios.

item	HB	HS	HP	HFL	HBL	HFK	HBK
AVG	61.52	45.86	48.09	36.84	37. 44	17.34	18..45
SD	1.59	1.62	1.99	1.71	1.21	0.72	0.62
item	HFH	HSH	LFH	LSH	LBH	LI	LCC
AVG	12.33	11.44	16.78	17.34	8.15	52.13	20.48
SD	1.45	0.98	0.49	0.50	1.11	1.78	4.45

The actual body data (ABD) of the bull camel was obtained by manually using a metre ruler.

Estimation of the actual body data of the bull camel from the photo data

Assuming the scale of the actual body size measurement of the bull camel is λ, then the actual body data (XABD) of a certain parameter can be estimated based on the corresponding photo data (XPD)

$$X_{ABD} = \lambda X_{PD} \quad (1)$$

In present study λ=2.87.

Ratio analysis of the actual body data and the photo data

Ratio is an important mathematical concept, expressed by the quotient of two variables with the same unit. As shown in Fig 2, assuming that the total length of a line is AB, select a point C and divide the line AB into two parts AC and CB, if the ratio of AB to AC and the ratio of AC to CB is exactly 1.618, then call the point C is the golden section point of the line AB, and ratio 1.618 is called the golden ratio, marked as φ (Gary 2018; Pearce, 2020; Thapa and Thapa, 2018).

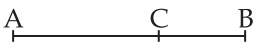

Fig 2. Golden-section

$$\frac{AB}{AC} = \frac{AC}{CB} = 1.618 \quad (2)$$

Optimisation of Bactrian camel weight formula

When evaluating the growth indicators of the Bactrian camel, the weight measurement of the Bactrian camel is a difficult task, so the weight of the camel is generally estimated by some regression empirical formulas, such as equation (3) (Tian *et al*, 1987).

$$Y(kg) = -773.57 + 2.66X_{ABD}(cm) + 4.06Z_{ABD}(cm) \quad (3)$$

where Y, X_{ABD} and Z_{ABD} are the weight, the actual body length and the actual chest circumference of the bull camel, respectively. Perform equivalent transformation on formula (3), that is, first divide the right side by X_{ABD}, and then multiply by X_{ABD}, and get the following formula

$$Y(kg) = -773.57 + (2.66 + 4.06Z_{ABD}/X_{ABD})\, X_{ABD}\ (cm) \quad (4)$$

Let's define actual chest circumference-to-actual body length ratio β_{ABD} as the quotient of the actual chest circumference Z_{ABD}(CC) of a Bactrian camel divided by its actual body length X_{ABD} (LI) , that is

$$\beta_{ABD} = CC_{ABD}/LI_{ABD} \quad (5)$$

$$Y(kg) = -773.57 + (2.66 + 4.06\beta_{ABD})\, \lambda\, LI_{PD}\ (cm) \quad (6)$$

In present study, β_{ABD}=1.571 and λ=2.87, so as long as the body length LI_{PD} is extracted from the photo of the bull camel, its weight can be estimated.

Results

The statistical average (AVG) and the standard deviation (SD) of the actual body data of Gobi red bull camel are shown in Table 1 and it was found that the dispersion of the actual body data of bull camels is relatively small.

Extracting the size of the object in the photo and calculating its actual size provide an effective technical basis for non-contact measurement (Adikari *et al*, 2017; Feng Tian, 2014) of certain animals that are difficult to capture or target placed in dangerous places.

In present study, a Canon E05-30D digital camera was used to collect the front view and side view photos of the 40 bull camels (Fig 3). Total of 20 side view photos with good viewing angles were selected, and the photo data (PD) were measured by Photoshop. The result of statistical analysis of photo

Fig 3. Bactrian camel photo: (a) Front view, (b) Side view.

data and ratios are shown in Table 2. The dispersion of the photo data of bull camel is smaller than that of the actual body data.

Table 3 shows the estimated data (ED) based on the photo data. The error between the estimated body data and the actual body data was not too

Table 3. Estimated body data based on the photo data (cm).

Parameter	HB	HS	HP	HFL	HBL	HFK	HBK
PD	61.52	45.86	48.09	36.84	37. 44	17.34	18..45
ED	176.56	131.62	138.02	105.73	108.45	49.77	52.95
ABD	176.30	127.48	135.13	106.33	106.48	46.88	51.73
Error (%)	0.15	3.20	2.12	0.57	0.91	5.98	2.33
Parameter	HFH	HSH	LFH	LSH	LBH	LI	LCC
PD	12.33	11.44	16.78	17.34	8.15	52.13	20.48
ED	35.39	32.83	48.16	49.77	23.39	149.61	58.78
ABD	35.53	33.03	43.35	53.18	23.68	151.40	240.03(CC)
Error (%)	0.14	0.20	10.51	6.62	0.29	1.189	4.91**

**Note: CCABD-4.08* LCCED=4.91

Table 4. Ratios from actual body data and photo data.

Parameter	HB/HFL	HFL / HB- HFL	HS- HFK / HFK	HS/HS-HFK	HH1/HS	HS/ HH1-HS
ratioABD	1.658	1.520	1.719	1.582	1.662	1.511
ratioPD	1.669	1.493	1.645	1.608	1.610	1.638
Parameter	HB/HBL	HBL/ HB-HBL	HP-HBK/HBK	HP/HP -HBK	HH2/HP	HP/ HH1-HP
ratioABD	1.656	1.524	1.612	1.620	1.549	1.762
ratioPD	1.643	1.556	1.607	1.622	1.517	1.867
Parameter	LWH**/LFH+LBH	LFH+LBH /LSH	HFK/HS	LFH/ LWH	HB-HFL /HB	HFK/ HH1- HFK
ratioABD	1.793	1.260	0.368	0.361	0.397	0.284
ratioPD	1.696	1.438	0.378	0.397	0.401	0.307
Parameter	LWH/LSH+LBH	LSH+LBH /LFH	HBK/HP	LSH/ LWH	HB-HBL /HB	HBK/HH2- HBK
ratioABD	1.563	1.773	0.383	0.442	0.396	0.328
ratioPD	1.658	1.519	0.384	0.410	0.391	0.338
Parameter	HB/HS	HH1/ HH1- HFK	HS/HFL	HH1**/HB		
ratioABD	1.383	1.284	1.199	1.202		
ratioPD	1.341	1.307	1.245	1.200		
Parameter	HB/HP	HH2/ HH2-HBK	HP/HBL	HH2**/HB		
ratioABD	1.305	1.378	1.269	1.187		
ratioPD	1.335	1.338	1.284	1.186		
Parameter	HH1-HFL/HFL	LFH /LSH	WFP/WH P	LFP/LHP	HFL/HBL	WS/ WP
ratioABD	0.992	0.815	1.185	1.113	0.999	1.092
ratioPD	1.005	0.968	—	—	0.984	—
Parameter	HH2- HBL/HBL	HFH/FSH	WFP/ LFP	WH P /LHP	HFK/HBK	HS/HP
ratioABD	0.966	1.076	1.00	0.94	0.906	0.943
ratioPD	0.949	1.078	—	—	0.940	0.954
Parameter	HH1/HH2	HH1/HFL	HFL/ HFK	HH1/HH1-HFL	HH1/HFK	HB/ HFK
ratioABD	1.012	1.992	2.268	2.013	4.519	3.760
ratioPD	1.012	2.005	2.125	1.995	4.259	3.547
Parameter		HH2/HBL	HBL/ HBK	HH2/ HH2-HBL	HH2/HBK	HB/ HBK
ratioABD		1.966	2.058	2.035	4.047	3.408
ratioPD		1.949	2.029	2.054	3.954	3.334

**Note: HH1= HFH+HB; HH2= HSH+HB; LWH= LBH+LFH+LSH

large, so this has important guiding significance for the non-contact measurement of the Bactrian camel body size.

In Table 3, the error between estimated body data and actual body data of bull camel was small, except for the length error of the first hump, which was 10.51%. It is considered as the larger error and is caused by the differences in the measuring position. In addition, CCABD/LCCPD=4.08λ, which means that the three-dimensional chest circumference of bull camel is as 4λ times as the line chest circumference on bull camel photo.

Considering the ratio combinations of all horizontal line segments and vertical line segments with visual segmentation significance, the following 105 vertical (the heights) ratios and horizontal (the lengths) ratios, 55 ratios belong actual body data and 50 ratios belong photo data, were calculated in bull camel body size data.

It can be seen from Table 4, the actual body data ratios and the corresponding photo data ratios have very similar values, and have interesting ratios, such as φ, 2φ, 1-1/φ, 2-1/φ, 1, 2, 4, and their statistical analysis results are shown in Table 5.

In Table 5, there are 32 ratios (16 for ABD data and 16 for PD data) with a mean of 1.611 which is very close to the golden ratio φ and their mean square error (MSE) is 0.013. There are also 16 ratios close to 1-1/φ, 8 ratios close to 2-1/φ, 8 ratios close to 2/φ, and 25 ratios close to 1, 8 ratios close to 2, 8 ratios close to 4, having relatively small MSE. Moreover, the dispersion of the mean of all ratios was very small.

Table 5. Statistical analysis of various ratios.

	Ratio values								AVG	SD	MSE
φ:	1.658	1.520	1.719	1.582	1.662	1.511	1.656	1.524	1.611	0.112	0.013
	1.669	1.493	1.645	1.608	1.610	1.638	1.643	1.556			
	1.612	1.620	1.549	1.762	1.793	1.260	1.563	1.773			
	1.607	1.622	1.517	1.867	1.696	1.438	1.658	1.519			
1-1/φ:	0.368	0.361	0.397	0.284	0.383	0.442	0.396	0.328	0.373	0.039	0.002
	0.378	0.397	0.401	0.307	0.384	0.410	0.391	0.338			
2-1/φ	1.383	1.284	1.341	1.307	1.305	1.378	1.335	1.338	1.334	0.033	0.003
2/φ:	1.199	1.202	1.245	1.200	1.269	1.187	1.284	1.186	1.222	0.036	0.002
1	0.992	0.815	1.185	1.113	0.999	1.092	0.999	1.092	1.000	0.076	0.006
	1.005	0.968	—	—	0.984	—	0.984	—			
	0.966	1.076	1.00	0.94	0.906	0.943	1.012				
	0.949	1.078	—	—	0.940	0.954	1.012				
2	1.992	2.268	2.013	1.966	2.058	2.035			2.041	0.0082	0.008
	2.005	2.125	1.995	1.949	2.029	2.054					
4	4.519	3.760	4.259	3.547	4.047	3.408	3.954	3.334	3.854	0.391	0.175

Discussion

It is evident from Table 1 and Table 2 that the body size data of Gobi Red Bull camel possess a small dispersion, which means that the bull camels selected by the herders based on long-term nomadic production experience (Dongjirzhab, 2019) are more consistent in appearance and size, and it indicates that the data analysis method used in this study is relatively stable. As shown in Table 3, the error between the actual body data of bull camel and estimated body data based on bull camel photos is small enough to prove the reliability of the calculation method of extracting actual body size from photos, can provide a theoretical basis for the non-contact measurement of Bactrian camel body size measurements. The golden ratio φ has many magical characteristics, such as $\varphi=1+1/\varphi$, $\varphi^2=1+\varphi$ and so on (Gary, 2018; Thapa and Thapa, 2018). In Table 5, there are 32 ratios are very close to the golden ratio φ, and 32 ratios contain 1/φ. The appearance of these golden ratios φ may not be accidental, and may indicate the natural mystery inherent in the body morphology of the Bactrian camel.

Conclusion

In present study, 21 actual body size parameters and corresponding photo data of bull camel were measured. The error between the estimated body data from the photo data and the actual body was relatively small. The three-dimensional chest circumference of bull camel is 4λ times as the line chest circumference of bull camel photo. Many related golden ratio φ was found in Bactrian camel body size

data ratio. And an optimised formula for estimating the weight of bull camel based on photo data was derived.

In short, the bull camels selected by the herders based on long-term nomadic production experience are more consistent in appearance and size, and the calculation method of extracting actual body size from photos is reliable. It can provide a theoretical basis to the non-contact measurement of Bactrian camel body size and weight.

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COMPUTED TOMOGRAPHIC IMAGING OF EYE OF THE DROMEDARY CAMEL

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ABSTRACT

Three cadaver heads of adult camels were scanned by 16 slice CT scan machine. The CT imaging findings of ocular components were recorded. The eyeball appeared as hypoattenuating structure whereas, the lens appeared as a hyperattenuating round structure in the centre of the globe. The bony limits of the orbital cavity, ocular and peri-ocular tissues were well identified in axial and reconstructed CT images. CT provided detailed information of the bony orbit.

Key words: Cadaver head, camel, computed tomography, dromedary eye

Computed tomography (CT) is now extensively used in veterinary medicine for understanding and diagnosing a variety of diseases (Pollard and Puchalski, 2011). Many researchers have done CT studies in camels to understand the anatomical details of various body parts. CT of the eyes (Abedallah *et al*, 2017), brain (Blanco *et al*, 2015), temporomandibular joint (Arencibia *et al*, 2012), tarsus (Hagag *et al*, 2013), metatarsus and digits (El-Shafey and Kassab, 2013), hind limbs in healthy dromedary camel foot (Elnahas *et al*, 2015), arthrography of carpus (Badawy, 2016 and Badawy *et al*, 2016) and structures of the nasal cavity, paranasal sinuses, oral cavity, orbit, and cranium (Alsafy *et al*, 2014) have been done in recent decade.

CT provides cross-sectional and three-dimensional images of the eye and orbital cavity (Dennis, 2000 and Smallwood *et al*, 2002) and different tissues are visualised with good anatomic resolution, high contrast and also possible to scan from different tomographic planes of the body. The cross-sectional anatomy of the dromedary camel eye could be useful in the evaluation of different conditions like congenital diseases, fractures, tumours, etc. The present study was aimed to describe normal anatomical details of the camel eye using CT imaging.

Materials and Methods

Three adult cadaver camel heads without ocular pathology were collected immediately after the death for the CT scanning using 16 slice CT scan machine (Supria, Hitachi, Ltd). The head was positioned on table with the rostral side directed towards the gantry (Fig 1). Axial CT images were obtained using 120 KV, 100-350 mAs and 5 mm slice thickness. The appearance of orbit and ocular contents were initially recorded in axial plane and later reconstructed as multiplanar and 3-D images were also obtained. The orit and the globe were examined in bone window (Window Width, WW = 2000, Window level WL = 200) and soft tissue window (WW = 300, WL = 40), respectively.

Results and Discussion

The present study documents that anatomy of the eye by CT images. These CT images delineated the dromedary eye and surrounding periorbital structures. Similar results have been observed in llamas by Hathcock *et al* (1995). CT images provided good discrimination between bone and soft tissue structures of the orbit. Images were obtained in the axial plane and their multiplanar reconstructed images. The osseous structures of the orbit were visible as hyperattenuating structures. The orbit was visualised well in axial and 3-D reconstructed CT images. The globe was completely circular and encircled by orbit. The margins of the bony orbit were formed by the frontal bone dorsally and its zygomatic process caudally, zygomatic bone ventrally and lacrimal bone rostrally (Fig 2). Anterior chamber, eye lens, vitreous chamber, peri-orbital fat, ocular muscles and optic nerve path were evident in both axial (Fig 3(A)) and dorsal planes (Fig 3(B)) of CT images. The eyeball appeared as hypoattenuating structure whereas the lens appeared as a hyperattenuating

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round structure in the centre of the globe. The aqueous and vitreous chambers were visualised as being filled with fluid attenuating densities cranial and caudal to lens, respectively. The ocular muscles were visualised as hyperattenuating bands intercalated by fat and optic nerve which travels in the centre of the muscle towards the optic canal. The optic nerve path was visualised in the dorsal plane of the CT image.

CT scanning images provide better peri-ocular details than images from the other imaging modalities. CT imaging was performed on cadaver heads immediately after the death of the camel to avoid or minimise the post mortem changes during CT imaging which was in agreement with Blanco *et al* (2015). In present study the eye lens, vitreous body

Fig 1. Positioning of cadaver camel head for CT scan.

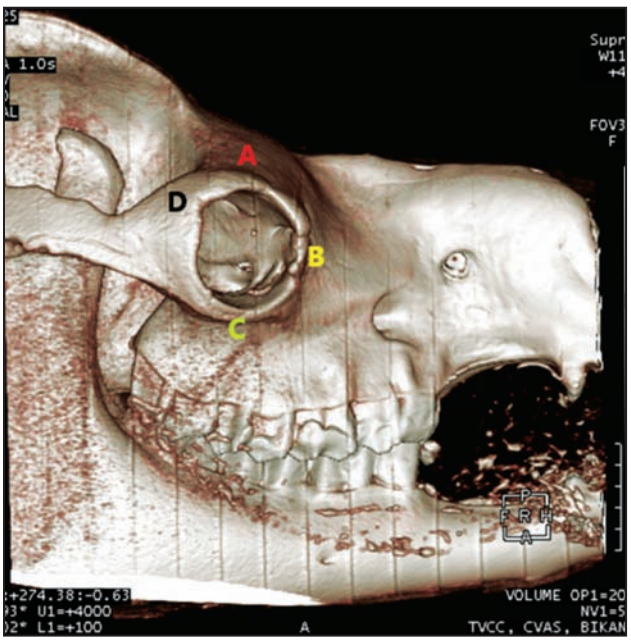

Fig 2. 3 D reconstructed CT image of camel head demonstrating bony orbit (right side). A-Frontal bone, B-Lacrimal bone, C-Zygomatic bone, D-zygomaticprocess of frontal bone.

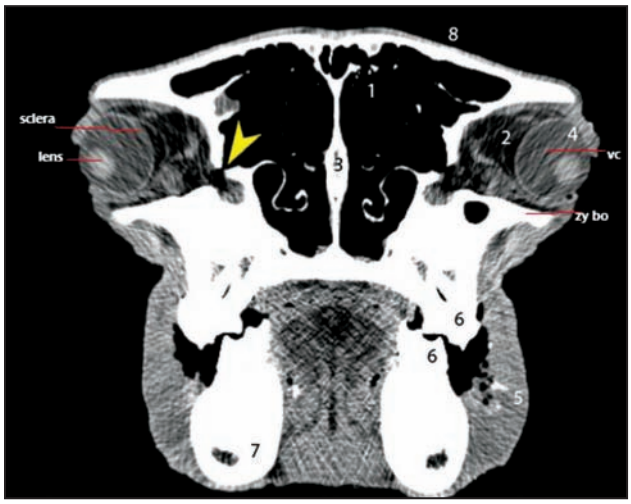

Fig 3A. Reformatted transaxial CT image of camel head demonstrating ocular structures. 1- Ethmoidal labrynth; 2- periorbital fat/tissue; 3-perpendicular plate of ethmoid bone; 4- eyeball; 5- masseter muscle; 6-upper and lower 5th cheek tooth; 7-molar part of mandible; zy bo-zygomatic bone; arrowhead – communication with nasal cavity

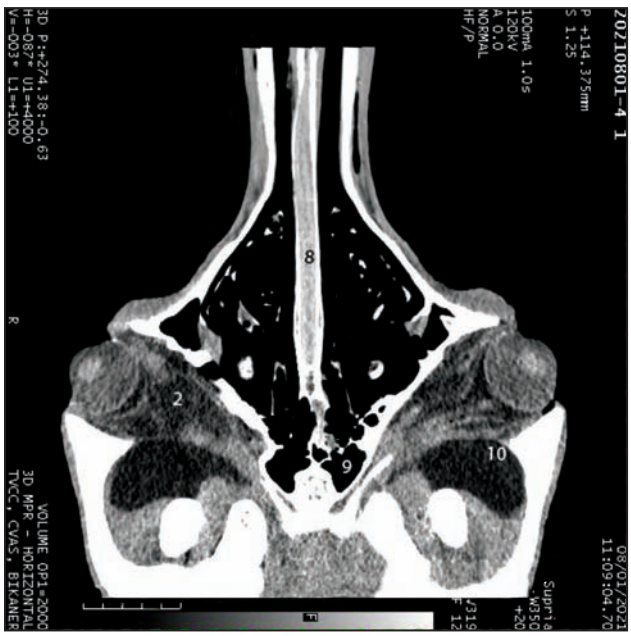

Fig 3B. Reformatted CT image in dorsal plane of camel head demonstrating ocular structures. 8- Interfrontal septum; 9- sphenoidal sinus; 10-maxillary sinus.

of eye, anterior chamber, and muscles of eye and periorbital fat were evident on the CT images and similar results were documented by Alsafy *et al* (2014) for dromedary eye.

CT allowed good visualisation of the anterior eye chamber, lens, scleral ring, posterior ocular wall, retrobulbar space, ocular nerve, and entire skull but was an inadequate technique for examining the cornea and uvea. The retrobulbar fat provided excellent image contrast that enabled easy visualisation of the extraocular structures and cortical bone (Daniel and Mitchell, 1999). Technical parameters (kV, mA) used in the present study were in agreement with that used for CT imaging of the head in camels (Arencibia *et al*, 2012; Blanco *et al*, 2015; Emam *et al*, 2020) and equines (Morrow *et al*, 2000; Solano and Brawer, 2004). The appearance of the lens, orbit, and globe during CT imaging was found similar to those reported previously by other researchers (Abedellaah *et al*, 2017). Anterior chamber, eye lens, vitreous chamber, peri-orbital fat, ocular muscles and optic nerve path were evident in both axial and dorsal plane of CT images which was in agreement with results of Solano and Brawer (2004) and Abedellaah *et al* (2017).

The present study describes the normal appearance of the eye of dromedary camels and can be used as reference later for future research.

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COXIELLA BURNETII IN DROMEDARY CAMELS: A POSSIBLE THREAT

Coxiella burnetii–induced human Q fever is one of the most widespread neglected zoonosis. The main animal reservoirs responsible for *C. burnetii* transmission to humans are domesticated ruminants, primarily goats, sheep, and cattle. The most recent *C. burnetii* serosurvey studies conducted in herds and farms in Africa, North Africa, Arabian Peninsula, and Asia highlighted that seroprevalence was strikingly higher in dromedary camels than in other ruminants. The *C. burnetii* seroprevalence in camel herds can reach more than 60% in Egypt, Saudi Arabia, and Sudan, and 70 to 80% in Algeria and Chad, respectively. The highest seroprevalence was in female camels with a previous history of abortion. Moreover, *C. burnetii* infection was reported in ticks of the *Hyalomma dromedarii* and *Hyalomma impeltatum* species collected on camels. Because of possible long-term persistence of *C. burnetii* in camel hump adipocytes, this pathogen could represent a threat for herds and breeding farms and ultimately for public health. Because this review highlights a hyperendemia of *C. burnetii* in dromedary camels, a proper screening of herds and breeding farms for *C. burnetii* is urgently needed in countries where camel breeding is on the rise. Moreover, the risk of *C. burnetii* transmission from camel to human should be further evaluated.

(Source: Devaux CA, Osman IO, Million M and Raoult D (2020) *Coxiella burnetii* in Dromedary Camels (*Camelus dromedarius*): A Possible Threat for Humans and Livestock in North Africa and the Near and Middle East? Front. Vet. Sci. 7:558481. doi: 10.3389/fvets.2020.558481)

THE WORLD'S FIRST CAMEL MILK ATM INSTALLED IN NAIROBI

A new shopping mall in Jumaira Centre in South C along ole shapara Road, Nairobi, Kenya has set up Camel Milk ATM at the entrance. It is the first vending machine of its kind. The love for camel milk is ever increasing in Africa, especially the Horn of Africa as the region is the emerging hub of camel milk for the young Africans. JUMAIRA CENTER, a new shopping center in Nairobi placed Camel Milk ATM at the enterance of the shopping center to attract customer and sell camel milk at affordable price as low as ksh. 10 Kenya Shillings equivalent to 10 cent USD.

WILL THE CAMEL CHEESE AND OTHER MILK PRODUCTS BE AVAILABLE IN SUPERMARKET?

The increase in demand for camel milk and products is not only translated into camel demography progress around the world, but also into multiplication of events as virtual scientific meeting (recently in Iraq, India, Pakistan, Kazakhstan, Morocco, Kenya, Sudan…), development projects and practical training in the dairy sector. The demand is coming from private camel dairy plants as well as from national or international institutions as European Union or FAO. Based on their experience in Saudi Arabia for processing camel milk into cheese, fermented milk and other milk beverages, Dr Bernard Faye (France) and Gaukhar Konuspayeva (Kazakhstan) have organised for the last 4-5 years several practical training on this topic, for example, in Mauritania and Chad with the support of FAO, at Laayoune in South-Morocco on the side-lines of the ISOCARD conference, at El-Oued in Algeria and Incirliova in Turkey in the frame of CAMELMILK project (supported by European Union), even in Canary Islands at Fuerteventura and at Montpellier in France (in CIRAD) where some farmers have switch their cattle to camel and aim to start milk and cheese production. Regular demands are coming for other countries as Kazakhstan, Niger, or Oman. Thanks to this enthusiasm for dairy products, we could expect to find camel cheese in supermarket for the next decades?

(Source: B. Faye)

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DIVERSITY OF BACTERIA AND FUNGI IN THE PREPUCE OF CAMELS (*Camelus dromedarius*)

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ABSTRACT

A total of 42 infertile male dromedary camels (4-12 years old, 500-800 kg) and 7 young male camels (control, 2-2.5 years old) were enrolled in the present study. All camels were sedated and preputial swabs were collected and immediately immersed in 1 ml sterile solution of 0.9% NaCl. Preputial swabs were transferred refrigerated within 1 hour to the bacteriological laboratory. After preputial swabbing, semen samples were collected from infertile camels using an electro-ejaculator and evaluated for routine semen quality parameters using the conventional methods. Statistical analyses were conducted by Student's *t*-test and Chi-square (X^2). Results revealed that semen parameters were reduced in infertile camels with preputial contamination. The bacterial count in 1 ml swab sample was $148x10^3$ and $0.24x10^3$ in infertile mature camels and control immature camels, respectively. Nine bacterial species isolated from 49 camels' preputial swabs comprised 41 gram-positive species (83.7%) and 8 gram-negative species (16.3%). The swabs of infertile camels had colonies species of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *E. coli*, *Pseudomonas aeruginosa*, *Actinomyces*, *Fusobacterium*, *Clostridium perfringens* at percentages of 35.72% (n=15), 21.43% (n=9), 11.91% (n=5), 9.52% (n=4), 7.14% (n=3), 4.76% (n=2), 4.76% (n=2), 2.38% (n=1), 2.38% (n=1), respectively. *Candida* colonies appears in concurrence with bacterial colonies in 28.57% (n=12). In control camels, the examined samples had colonies of *Streptococcus* spp., *Bacillus* spp. and *E. coli* spp. at proportions of 42.86% (n=3), 42.86% (n=3), 14.28% (n=1), respectively. *Candida* spp. observed in 5 colonies (71.43%) of control camels. In conclusion, *Staphylococcus*, *Streptococcus* and *Bacillus* were the frequently isolated bacterial spp. from prepuce of camel.

Key words: Bacterial diversity, camels, fungus, prepuce

The reproductive efficiency of camels under natural conditions is generally considered as low (Al-Qarawi, 2005) and genital infection is considered as most important reason (Tibary *et al,* 2006). It has been demonstrated that the preputial sac can act as a reservoir of organisms and it thus responsible for causing ascending uro-genital infection (Agartan *et al,* 2005). The preputial microbial community plays a key role in maintaining health and altered microbial communities have been associated with a variety of reproductive diseases (Nelson *et al,* 2010; Sandal and Inzana, 2010; Chaban *et al,* 2012). The source of camel preputial contamination is usually soil, faeces and female genital tract (Wickware *et al,* 2020), and microorganisms gain entry through preputial orifice (Paray *et al,* 2018). The camel often does not show any clinical signs of contamination, but infection is traced back through symptoms shown in female camels it has mated (Al-Qarawi, 2005). A long duration of semen collection and collection in a sitting position (El-wishy, 1988) increase the risk of contamination of prepuce of camel Bacterial contamination leads to a series of alterations including reduced sperm motility, morphology, various semen quality parameters (Najee *et al,* 2012; Perumal *et al,* 2013), and subsequent reduced fertility (Ochsendrof and Fuchs, 1993; Griveau *et al,* 1995). This study investigates the frequency of isolated bacteria and fungi in camels' prepuce.

Materials and Methods

Experimental animals

A total of 42 infertile male dromedary camels (4-12 years old, 500-800 kg) admitted to the

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Veterinary Teaching Hospital, King Faisal University and 7 young male camels (control, 2-2.5 years old) belonging to the Camel Research Centre, King Faisal University (25°23′N 49°36′E) were selected in the present study. The control males had no contact with females before or during the experiment (5 months) between November 2020 to and March 2021.

Samples collection

Infertile camels were restrained in sternal position with ropes, sedated intravenously with a combination of xylazine (Rompun®, Bayer; 0.15 mg/kg) and ketamine (Alfasan® 10%, Holland; 2.5 mg/kg) (White *et al,* 1982). Then, sedated camels were turned to lateral recumbency and their prepuce was cleaned externally with sterile saline solution (0.9% NaCl). A sterile swab was inserted and rotated in the prepuce, and immediately immersed in a sterile 5 ml plastic tubes containing 1 ml sterile physiological saline solution (0.9% NaCl). Preputial swabs were collected from the control males as in the infertile camels. All collected swabs were transferred refrigerated within 1 hour to the bacteriological laboratory in the Ministry of Agriculture, Al-Ahsa, Kingdom of Saudi Arabia. After preputial swabing, semen samples were collected from all infertile camels using an electro-ejaculator (Tingari *et al,* 1986). Semen samples were evaluated for routine semen quality parameters such as volume (mL), viscosity (Ghoneim *et al,* 2010), pH, per cent motile sperm, sperm concentration ($x10^6$/ml), and per cent sperm abnormalities (Table 1) by the same trained researcher using Sperm Vision 3.5 (Minitube of America, Inc.).

Table 1. Semen parameters of infertile mature camels (mean ± SEM; range).

Semen parameters	Infertile mature camels (n=42) (Range)	Reference*
Volume (ml)	6.32 ± 0.74 (7 – 25)	6.56 ± 1.73
Viscosity (0 – 5)	3.57 ± 0.25 (0 – 5)	4.25 ± 0.37
pH	8.11 ± 0.06 (6.8 – 8.4)	7.84 ± 0.14
Motility (%)	8.00 ± 2.11 (0 – 20)	43.13 ± 9.77
Sperm concentration ($x10^6$/ml)	37.07 ± 13.41 (1 – 100)	366.15 ± 47.55
Sperm abnormalities (%)	50.54 ± 4.65 (15 – 80)	26.00 ± 2.95

*Adapted from Waheed *et al* (2015).

Microbiological evaluation

Bacterial identification

Each swab sample was plated on 5% sheep blood agar, Mac-Conkey agar and *Salmonella-Shigella* agar and incubated at 37°C in 5% CO_2 for 24 h. Growing colonies were examined with Gram staining methods. Suspected colonies were identified with biochemical and carbohydrate fermentation tests using bioMérieux's API identification products (Chesbrough, 2004).

Counting colony forming units per millilitre (CFU/ml) of sample

The quantitative estimation of the microbial contamination of prepuce was performed by the standard plate count method (Shukla, 2011). An aliquot (0.05 ml) of the swab sample was diluted with 1:10 PBS (0.1M phosphate buffer containing 0.15M NaCl, pH 7.3), producing a serial-dilution. Plates of Columbia-blood-agar were inoculated with 100 µl of the diluted swab sample (101-105) and incubated at 37°C for 48 h. The bacterial colonies were counted with the help of a colony counter. The colonies were counted in relation to the swab sample aliquot of 1ml and expressed as CFU/ml.

Mycological examinations

Swab samples were examined for fungus and yeast by seeding the samples in Sabaroud's dextrose agar. The plates were incubated at 25°C for 5 days and checked for fungal growth from the fourth day onward. Fungal identification relied on the morphologic and physiologic features (de Hoog *et al,* 2000). *Yeasts* were sub-cultured to obtain pure cultures for identification. For definitive identification of *yeasts*, the carbohydrate assimilation pattern was defined by ID32 (Biomerieux, Bagno a Ripoli, Italy).

Statistical analysis

The data are presented as the means ± SEM and percentages. Analyses were conducted by Student's *t*-test and Chi-square (X^2) using INSTAT software 3.1 (2017).

Results and Discussion

The bacterial count in 1 ml swab sample was $148x10^3$ and $0.24x10^3$ in infertile mature camels and control immature camels, respectively (Table 2). Nine bacterial species isolated from 49 camels' preputial swabs comprised 41 gram-positive species (83.7%) and 8 gram-negative species (16.3%). The different types of colonies were *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Bacillus*

spp., *E. coli* spp., *Pseudomonas aeruginosa* spp., *Actinomyces* spp., *Fusobacterium* spp., *Clostridium perfringens* spp. and *Candida* spp. as shown in table 2. The swabs of infertile camels had colonies species of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *E. coli*, *Pseudomonas aeruginosa*, *Actinomyces*, *Fusobacterium*, *Clostridium perfringens* at percentages of 35.72% (n=15), 21.43% (n=9), 11.91% (n=5), 9.52% (n=4), 7.14% (n=3), 4.76% (n=2), 4.76% (n=2), 2.38% (n=1), 2.38% (n=1), respectively. *Candida* colonies appear in concurrent with bacterial colonies in 28.57% (n=12). In control camels, the examined samples had colonies of *Streptococcus* spp., *Bacillus* spp. and *E. coli* spp. at proportions of 42.86% (n=3), 42.86% (n=3), 14.28% (n=1), respectively. *Candida* spp. observed in 5 colonies (71.43%) of control camels (Table 2).

Table 2. Bacterial load, types and frequency in dromedary preputial samples.

Isolates	Infertile mature camels (N=42)	Control immature camels (N=7)
CFU/ml (mean ± SEM)	$148^{a} \pm 56.96x10^{3}$	$0.24^{b} \pm 0.12x10^{3}$
Staphlyococcus spp. (%)	11 (26.19%)	—
Staphlyococcus spp. + *Candida* (%)	4 (9.52%)	—
Streptococcus spp. (%)	5 (11.91%)	—
Streptococcus spp. + *Candida* (%)	4 (9.52^{a}%)	3 (42.86^{b}%)
Corynebacterium spp. (%)	5 (11.91%)	—
Bacillus spp. (%)	3 (7.14^{a}%)	2 (28.57^{b}%)
Bacillus spp. + *Candida* (%)	1 (2.38^{a}%)	1 (14.28^{b}%)
E. coli spp. (%)	3 (7.14%)	—
E. coli spp. (%) + *Candida* (%)	—	1 (14.28%)
Pseudomonas aeruginosa (%)	2 (4.76%)	—
Actinomyces spp. + *Candida* (%)	2 (4.76%)	—
Fusobacterium + *Candida* (%)	1 (2.38%)	—
Clostridium perfringens (%)	1 (2.38%)	—

Means and percentages with dissimilar superscripts in the same row are significantly different at P<0.05.

In the present study, semen parameters were reduced in infertile camels. These reduced values were accompanied with preputial contamination in these camels. This relationship indicates that preputial microbial load might be considered as a relevent cause of infertility in dromedaries. Previous studies achieved the same findings (Ochsendrof and Fuchs, 1993; Griveau *et al,* 1995; Najee *et al,* 2012; Perumal *et al,* 2013). Published data on the presence and distribution of bacteria and fungi in the camel prepuce are scarce. The current study revealed that the bacterial count in 1 ml semen was 0.24 and 148 $x10^{3}$ CFU in control immature and infertile camels, respectively. The young bulls had significantly lower total bacterial counts than older animals (Reddy *et al,* 1971). However, Brown *et al* (1974) found no correlation between the age and total bacterial count. The saprophytic flora of the prepuce of healthy bulls includes numerous bacterial species (Thibier and Guerin, 2000). The collected preputial samples were colonised by species of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *E. coli*, *Pseudomonas*, *Actinomyces*, *Fusobacterium* and *Clostridium*. Species of *Staphylococcus*, *Streptococcus*, *Corynebacterium* and *Bacillus* were the frequently isolated bacteria. Twelve bacterial spp. were identified in dromedary camels' prepuce that were *Serratia liquefaciens*, *Staphylococcus aureus*, *Streptococcus* spp., *Klebsiella ozaenae*, *Pseudomonas* spp., *Shigella* spp., *Enterobacter cloaceae*, *Flavobacterium* spp., *Actinomyces* spp., *Acinetobacter* spp., *Acinetobacter calcoaceticus* and *Bacillus* spp. (Serin *et al,* 2010). *Streptococcus* spp., *Pseudomonas* spp. and *Staphylococcus* spp. are the most common bacteria isolated from the uterus of infertile camels (Wernery and Wernery, 1992; Ali *et al,* 2010; Almohasen, 2011). Then, camel preputial contamination might lead to uterine infection and *vice versa*. It has been demonstrated that the camel prepuce can act as a reservoir of organisms and it thus might be responsible for infertility (Agartan *et al,* 2005). *Staphylococcus aureus* was the most frequently isolated aerobic microorganism in specimens obtained from the prepuce of dogs (Ling and Ruby, 1978). The present study was done during the camels' rutting season with with a marked peak in sexual activity. This activity might lead to preputial contamination (Zhao, 2000; Fatnassi *et al,* 2014). In the present study, *Candida* spp. was colonised in 26.5% of the collected samples. *Candida* is a common mycoflora present in the genitalia of healthy female camels (Shokri *et al,* 2010). In camels mating, the penis penetrates the cervical canal, and in some cases, it enters deep into the uterine cavity (Serin *et al,* 2010). During the camels' rutting season, the atmospheric concentration of fungus spores is twice as high as in the non-rutting season (Bartzokas, 1975). In stallions, *yeasts* were isolated in 9.1% of the preutial samples (Rota *et al,* 2011). In conclusion, species of *Staphylococcus*, *Streptococcus* and *Bacillus* were the frequently isolated bacteria from camels' prepuce. The present study recommends the washing of the camel's prepuce before mating.

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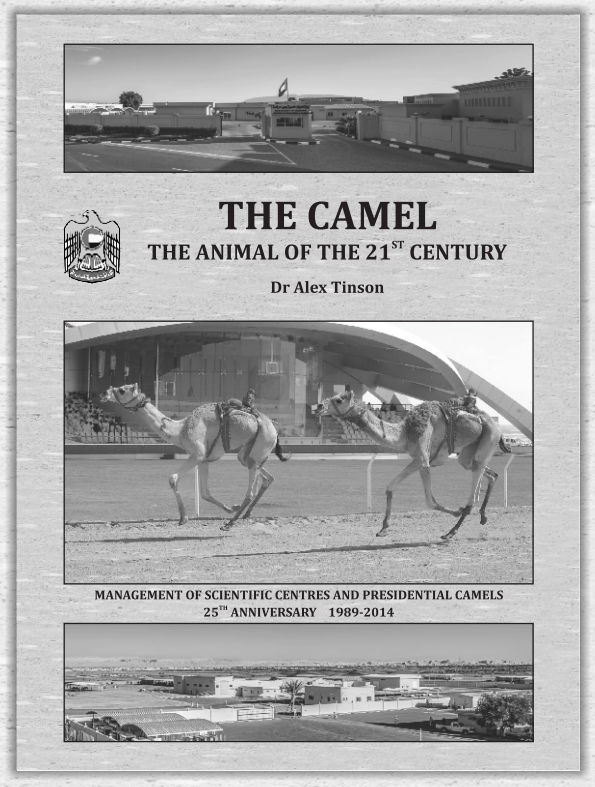
THE CAMEL

THE ANIMAL OF THE 21ST CENTURY

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



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EVALUATION OF BACTERIAL AND FUNGAL FLORA IN HEALTHY FEMALE REPRODUCTIVE TRACT OF CAMELS (*Camelus dromedarius*)

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ABSTRACT

Swab samples for microbial culture were obtained from the vaginae, cervices and uteri of 10 healthy fertile dromedary camels. Swab specimens were collected by use of a double-guarded uterine equine swab. Specimens were cultured for aerobic and anaerobic bacteria as well as fungus. The 90%, 50% and 30% of the swabs collected from the vaginae, cervices and uteri were contaminated, respectively. The difference between the rate of contamination was significant (P<0.05). Coagulase-negative *staphylococci*, *Staphylococcus aureus*, *Streptococcus* spp., *E. coli*, *Bacillus* spp. and *Proteus* spp. were isolated from the samples collected from the vaginae of camels. Coagulase-negative *Staphylococci*, *Streptococcus* spp. and *Bacillus* spp. were identified in the swabs collected from the cervices. Coagulase-negative *Staphylococci* and *Bacillus* spp. were isolated from the swabs collected from the uteri. Double and triple infections were only reported in the samples collected from the vaginae. No anaerobic bacteria could be isolated from the vaginae, cervices or uteri. *Candida albicans* and *Cryptococcus* were isolated from the vaginae. Only *Candida albicans* could be isolated from cervices and uteri. This study identified a great microbial population's diversity in the vagina which decrease toward the uterus. Coagulase-negative *Staphylococci* and *Bacillus* spp. were the dominant bacteria isolated from the vaginae, cervices and uteri.

Key words: Bacteria, camel, fungus, microflora, reproductive tract

Reproductive disorders cause economic losses through increased calving intervals loss of milk production and culling of valuable breeding animals (Bellows *et al*, 2002). Genital tract infections are the common cause of reproductive disorders in female camels (Ali *et al*, 2010; Tibary and Anouassi, 2001; Khalafalla *et al*, 2017). Knowledge of microbial communities colonising camel's healthy female genital tract may lead to improved treatment of infected ones with probiotics (El-Deeb *et al*, 2020). Data of genital microflora is helpful in understanding the pathological disorders that could be diagnosed in this species (Shokri *et al*, 2010). Recently, the microbial communities of the vagina have also been used as a fertility biomarker (Deng *et al*, 2019). Although the plenty of information considering the microbes colonising the infected reproductive tract (Elshazly *et al*, 2020; Khalafalla *et al*, 2017; Al-Humam, 2015; Mshelia, *et al*, 2014) and very limited studies describing the microbial community along the healthy female genital tract of camels is available. Nowadays, there is an increasing interest in the studies related to the microflora of the reproductive tract, and its relationship to disease and health (Appiah *et al*, 2020; Laguardia-Nascimento *et al*, 2015; Shokri *et al*, 2010). The present study was aimed to elucidate the microbial flora of the different segments of the reproductive tract in healthy female dromedary camels.

Materials and Methods

Animals

The Scientific Research Deanship Ethics Committee of King Faisal University approved this study (Contract # 216067). During breeding season (from November to April), 10 adult non-pregnant dromedary camels, aged 7 to 14 years were, kept in the Camel Research Centre, King Faisal University (25°23′ N 49°36′ E). These were apparently healthy with a sound history of fertility in the herd.

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To exclude the disorders of the genital tract each animal underwent breeding soundness examinations (Tibary and Anouassi, 1997). All camels were clinical examination by visual appraisal for any signs of abnormal vulval discharge, rectal palpation of the reproductive tract and ovaries, as well as trans-rectal ultrasonography using linear-array 5 MHz transducer (UST588U-5, SSD-500V, ALOKA, Co., Japan).

Procedures for collecting of microbiological smears

Before collection of the vaginal, cervical, or uterine microbiological samples, the external genitalia of camels were washed with warm water and soap, rinsed twice with 0.1% povidoneiodine, and dried with a clean cloth. Deep vaginal smears were collected under aseptic conditions using double-guarded uterine equine culture swabs (Equi-Vet uterine culture swab, Kruuse, Denmark). After introducing the swab into the vestibule, the swab was firstly directed almost dorsally before redirecting it cranially and then rolled over the cranial vaginal mucosa. The swab was retracted into the protecting tube of the double guarded swab and removed from the animal. For collecting a cervical microbiological sample, a sterile double-guarded swab was directed to the external cervical os guided by a gloved hand and then rotated 360°. The swab was then retracted into the protecting tube and removed from the camel. Uterine microbiological swabs were obtained by passing a sterile double-guarded swab through the cervix, guided by a gloved hand, and rotated 360° over the right and left uterine horn. Afterwards, the swab was retracted into the protecting tube. The swabs were transferred at ambient temperature in Amies' modified media to the laboratory and cultured within 8 h.

Bacterial isolation and identification

Each swab was inoculated on to Columbia agar (CM331; Oxoid Basingstoke, UK) supplemented with 5% citrated sheep blood, MacConkey agar (Oxoid, Basingstoke, UK). After inoculation, the plates were incubated under aerobic and anaerobic for 18 to 24 h at 37 °C and a further 24 h if bacterial growth had not conditions occurred. If > 90% of the colonies on an incubated agar plate belonged to one species, the result was considered as substantial growth in monoculture. Any other growth in mixed culture was considered the result of contamination and recorded as a negative growth result (Koneman *et al*, 2005). Isolate identification was confirmed by Vitek 2 technique (BioMerieux, Marcy L'Etoile, France).

Mycological identification

For mycological examination, samples were seeded on to Sabouraud glucose agar (Merck Co., Darmstadt, Germany) supplemented with an antibiotic (chloramphenicol; 0.005%). The plates were kept at 25°C for 7-10 days and inspected for fungal growth from the 4th day onward. The fungus was identified by examination of morphologic and physiologic features (deHoog *et al*, 2000). Yeasts were sub-cultured to obtain pure cultures for identification. For decisive identification of yeasts, the carbohydrate assimilation pattern was defined by ID32 (Biomerieux, Bagno a Ripoli, Italy).

Data analysis

All statistical analyses were performed using JMP SAS 11.0.0 (SAS Institute Inc., Cory, NC, USA) program. The results of vaginal, cervical and uterine culture are presented as percentages. The chi-square test was used to compare the differences between percentages. Differences of $P < 0.05$ were considered to be significant.

Results and Discussion

Table 1 showed the microbial community structures of the healthy female reproductive tract of camels. The results demonstrated that 90% (9/10), 50% (5/10) and 30% (3/10) of the swabs collected from the vaginae, cervices and uteri, respectively of the studied animals were infected. The difference between the groups was statistically significant ($P<0.05$). A total of 6 types of isolates were identified from the investigated animals. Microbiological investigation of the swab collected from the vaginae, indicated the growth of coagulase negative *Staphylococci*, *Staphylococcus aureus*, *Streptococcus* spp., *E. coli*, *Bacillus* spp. and *Proteus* spp. (six types of colonies). Cultures of the cervical swabs were colonised by coagulase-negative *Staphylococci*, *Streptococcus* spp. and *Bacillus* spp. (three types of colonies). Coagulase-negative *Staphylococci* and *Bacillus* spp. were isolated from the uterine swab (two types of colonies). Coagulase negative *Staphylococci* infected 44.4%, 20%, 20% and 70%, respectively of the swabs collected from vaginae, cervices and uteri. *Staphylococcus aureus* could only be detected in the vaginae (11.1%). Samples collected from the vaginae 22.2% and uteri 20%, were found positive for *Streptococcus* spp. *E. coli* (22.2%) and *Proteus* spp. could only be isolated from the vaginae. *Bacillus* spp. were characterised in the swabs collected from vaginae (55.6%), cervices (60%) and uteri (66.6%) of the studied camels. Single aerobic bacterial infection

was recorded in 44.4%, 100% and 100% in vaginae, cervices and uteri, respectively of studied animals. Double (44.4%) and triple (11.1%) aerobic bacterial infection were only recorded in samples collected from vaginae. No anaerobic bacteria could be detected by all cultured samples. Fungal infection could be isolated from samples of vaginae (40%), cervices (10%) and uteri (10%). *Candida albicans* and *Cryptococcus* were isolated from the vaginae, cervices and uteri of the studied animals. *Candida albicans* were isolated from 75% of the fungal infected samples swabbed from the vaginae and 100% of the fungal infected samples collected from cervices and uteri. *Cryptococcus* was only isolated from fungal infected samples swabbed from the vaginae (25%). Single fungal infection was recorded in 100% fungal infected swabs.

Table 1. Microbial community structures of female reproductive tract of healthy camels.

	Vagina	**Cervix**	**Uterus**
Number of infected animals	90%^{a} (9/10)	50%^{b} (5/10)	30%^{c} (3/10)
Number of isolates	6	3	2
No growth	10%^{a} (1/10)	50%^{b} (5/10)	70%^{b} (7/10)
Type of isolates			
coagulase-negative *Staphylococci*	44.4%^{a} (4/9)	20%^{a} (1/5)	33.3%^{a} (1/3)
Staphylococcus aureus	11.1% (1/9)	0.0%	0.0%
Streptococcus spp.	22.2%^{a} (2/9)	20%^{a} (1/5)	0.0%
E. coli	22.2% (2/9)	0.0%	0.0%
Bacillus spp.	55.6%^{a} (5/9)	60%^{a} (3/5)	66.7%^{a} (2/3)
Proteus spp.	11.1% (1/9)	0.0%	0.0%
Type of Infection			
Single infection	44.4%^{a} (4/9)	100%^{a} (5/5)	100%^{a} (3/3)
Double infection	44.4% (4/9)	0.0%	0.0%
Triple infection	11.1% (1/9)	0.0%	0.0%
Anaerobic bacteria contaminant			
Number of infected animals	0.0%	0.0%	0.0%
Fungal contaminant			
Number of affected animals	40%^{a} (4/10)	10%^{a} (1/10)	10%^{a} (1/10)
Number of isolates	2	1	1
Types of isolates			
Candida albicans	75%^{a} (3/4)	100%^{a} (1/1)	100%^{a} (1/1)
Cryptococcus	25% (1/4)	0.0%	0.0%
Type of Infection			
Single infection	100%^{a} (4/4)	100%^{a} (1/1)	100%^{a} (1/1)

Percentages with dissimilar superscript letters in the same row are significantly different at $P < 0.05$

Genital microflora has been discussed in different animal species such as horse (Scott *et al*, 1971; Hinrichs *et al*, 1988), cow (Messier *et al*, 1984; Otero *et al*, 1999; Otero *et al*, 2000), sheep (Moorthy and Singh, 1982), goat (Fasanya *et al*, 1987) and dog (Baba *et al*, 1983). There is scarce information regarding microbial composition in the dromedary camel's reproductive tract. The current results show significant differences between the number of infected swabs collected from the vaginae, cervices and uteri. Moreover, 6 types, 3 types and 2 types of colonies were identified in the swabs collected from vaginae cervices uteri, respectively. As the cervix and vaginae serve as a physical and immune barrier against pathogens passage into the uterine cavity (Källerö, 2010; Barrios De Tomasi *et al*, 2019; Di Paola *et al*, 2020), the minimum infected swabs were recovered from the uterus of the studied animals. As in mare (Scott *et al*, 1971; Barba *et al*, 2020) and cow (Messier *et al*, 1984), the current results showed that the uterine environment is not sterile. The microorganisms isolated from the genital tract of the studied camels were dominated by Coagulase-negative *Staphylococci* and *Bacillus* spp. Likewise, an abundance of Coagulase-negative *Staphylococci* and *Bacillus* spp. were isolated from healthy genitalia of cows (Amin *et al*, 1996; Otero *et al*, 1999; Otero *et al*, 2000; Giannattasio-Ferraz *et al*, 2019), ewes (Moorthy *et al*, 1982; Quinlivan, 1970) and dogs (Baba *et al*, 1983). *E. coli* and *Proteus* spp. were the two genera of the Enterobacteriaceae family that have been isolated from the vaginae of the studied animals. Laguardia-Nascimento *et al* (2015) reported that gut affects both the uterine and vaginae microflora. Moreover, Enterobacteriaceae, especially *E. coli* have been isolated from the urogenital tract of cattle (Torres *et al*, 1994; Otero *et al*, 2000). The biological niches in the host microflora are not disconnected environments but are a system of interrelated communities that are frequently exchanging (Neckovic *et al*, 2020). Although, *E. coli* considered as normal vaginal flora (Tibary and Anouassi, 2001), it could be isolated from cases of endometritis in camelids (Tibary *et al*, 2006). Wang *et al* (2013) reported that *E. coli* is isolated from both healthy cows and those with uterine infections, they were found in much greater numbers in infected cows. The vaginae of 11.1% of the studied animals were colonised with *Staphylococcus aureus*. This result is comparable with that recorded

in camels and cows by Mshelia *et al* (2014). This bacteria has been isolated in camels suffering from endometritis (Wernery and Kumar, 1994; Tibary and Anouassi, 2001; Ali *et al*, 2010). *Streptococcus* spp. were isolated from both vaginae and cervices of the camels enrolled in this study. *Streptococcus* spp. are frequently isolated from the vagina of cows (Otero *et al*, 1999, Otero *et al*, 2000; Zambrano-Nava *et al*, 2011), ewes (Moorthy and Singh, 1982; Manes *et al*, 2010) and mares (Hinrichs *et al*, 1988). In this study, no anaerobic bacteria could be detected by all samples cultured. Anaerobic bacteria have been isolated from cattle with endometritis (Lewis, 1997; Sheldon and Dobson, 2004; Galvão *et al*, 2019), metritis (Földi *et al*, 2006; Bicalho *et al*, 2012), and pyometra (Ruder *et al*, 1981; Olson *et al*, 1984). Mycological culture of swabs revealed that *Candida albicans* was the most common species isolated from the genital tract of the studied camels. Similarly, Shokri *et al* (2010) and Azarvandi *et al* (2017) recorded that *Candida* spp. as the main mycotic isolates of healthy camels' and horse's female genitalia, respectively. However, *Candida albicans* were isolated from 30.5% of cattle, buffalo, sheep and goat suffered from reproductive disorders (Osman and Gabal, 1978). Shokri *et al* (2010) isolated reported, *Cryptococcus* from the swabs collected from the uterus.

This study identified a great diversity in the vaginal microbial communities which decreased toward the uterus. Coagulase-negative *Staphylococci* and *Bacillus* spp. were common bacteria isolated from the vaginae, cervices and uteri.

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INTERNATIONAL SEMINAR ON PROMOTION OF CAMEL MILK VALUE CHAIN: TECHNOLOGY TRANSFER AND PUBLIC-PRIVATE PARTNERSHIP (PPP)- CAMILK 2022

International seminar on Promotion of camel milk value chain: Technology transfer and public-private partnership (ppp)- Camilk2022 shall take place from June 1-3, 2022 at Zarzis, Tunisia. There will be three themes. The theme 1 will cover breeding and camel milk production, livestock building, nutrition, health, dairy potential, milking mechanisation. Theme 2 will cover camel milk technology, camel milk technologies and processing, from milk storage to dairy product and Theme 3 will cover camel milk value chain: management, organisation, governance, promotion, development and innovation. Other details can be seen on https://camilk2022.com/

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SELECTED HEAVY METALS AND THEIR RISK ASSESSMENT IN CAMELS (*Camelus dromedarius*)

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ABSTRACT

The study was carried out to determine the residual levels of heavy metals (zinc, iron, copper, lead, and cadmium) in tissues (meat, liver and kidney), serum and hair of 3 camel breeds (Magaheem, Maghateer and Wadha) collected from Al-Omran slaughterhouse, eastern province, Saudi Arabia by using Atomic Absorption Spectrometer. Camel breed influenced Zinc (Zn) accumulation and distribution in organs, muscle, and arranged in descending manner as follows: hair> liver> muscle > kidney> serum. The iron content in all male camel samples was considerably greater than in female camel. Furthermore, significant strong positive correlation between muscle and serum iron was established. All examined samples contained copper (Cu), the highest value was 17.78 ± 0.85 mg kg^{-1} detected in liver samples of Maghateer breed. The descending manner of Cu as follows: liver > muscle > hair > kidney > serum. In addition, the female liver contained significantly higher Cu than the male liver. Lead (Pb) residue was detected in all examined samples among different breeds except muscle samples of Maghateer and Magaheem breeds. The cadmium (Cd) values ranged from 0.0001 mg kg^{-1} in the muscle of the Maghateer breed to 4.5113 mg kg^{-1} in the hair of the Wadham breed. The meat and offal of all examined breeds contained lower Pb and Cd levels than the maximum permissible limit. The estimated daily intake (EDI) due to consumption of camel meat below the tolerable daily intake (TDI). In addition, the hazard ratio (HR) and hazard indices (HIs) values were far below one for adults.

Key words: Camel, heavy metals, metal toxicity, risk assessment, tissues

Metals like zinc (Zn), copper (Cu), and iron (Fe) are essential elements for maintaining proper bodily processes and blood synthesis. Metals like lead (Pb), cadmium (Cd), and arsenic (As) detected in contaminated food from both animal and plant sources are considered as toxic. Consequently, for both food safety and human health, monitoring the concentrations of these elements in human food is critical (Bortey-Sam *et al,* 2015; Hassan *et al,* 2020).

Heavy metal contamination in the environment has primarily been caused by natural geology or anthropogenic industrial sources, such as cadmium (Cd) and lead (Pb) (Van der Voet *et al,* 2011). Heavy metal pollution is regarded as one of the most serious issues since these metals cannot be degraded and so remain in the environment indefinitely (Baykov *et al,* 1996). Heavy metals are considered a dangerous environmental pollutant due to their ability to enter the food chain as well as their cumulative effect as residues (Asli *et al,* 2020). Although human exposure to these elements during meat consumption rarely leads to severe poisoning, their accumulation in the body may have negative effects on health (Chen *et al,* 2013).

Many investigators found the heavy metals in muscle, edible offal (liver and kidney) and serum of camel carcasses (Eltahir *et al,* 2010; Badis *et al,* 2014; Khalafalla *et al,* 2015; Meligy *et al,* 2019; and El-Ghareeb *et al,* 2019). Determining heavy metal residues in meat and edible offal is a very important issue to protect consumers in Saudi Arabia, as well as measuring these residues in camel hair to investigate the extent of environmental pollution.

The current research was aimad to determine the levels of toxic metals (Pb and Cd) as well as the necessary elements (Zn, Cu, and Fe) in camel meat, serum, hairs and offals. The metals-dietary intake and health risk assessment were also determined from public health point of view.

Materials and Methods

Collection of Samples

A total of 225 tissue samples (muscles, liver, and kidney), serum, and hair samples (n = 75) were

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taken from three local camel breeds at random from the Al-Omran central slaughter house in Saudi Arabia from November 2020 to February 2021. Age of the animals was < 5 to > 10 years. The sampled animals were apparently healthy. Samples were collected in plastic falcon tubes and kept at -20°C until these were extracted and measured.

Sample preparation and extraction

The Shimadzu AA-7000 Atomic Absorption Spectrophotometer (Japan) was used in conjunction with Flame Atomic Absorption Spectrometry (FAAS) and a graphite furnace atomic absorption spectrometry system (GFAAS) to assess the amounts of the trace elements Fe, Zn, Cu, Cd, and Pb. In addition, air/acetylene gas (10:1.5) was employed in FAAS.

For the analysis of Cu, Fe, and Zn, flame atomic absorption was applied whereas, Cd and Pb were measured using GFAAS system (argon being an inert gas). The Shimadzu ASO6100 Automatic Sampler was used to inject the samples into the GFAAS and FAAS. The Mars Xpress Microwave Digestion System (CEM Cooperation, Mathews, North Carolina, USA) was used to digest the samples. Polytetrafluoroethylene vessels were used for all digesting procedure. Before each digestion step, the containers were washed with 5 ml concentrated nitric acid. External calibration was used to conduct a quantitative examination of the samples. In polytetrafluoroethylene digesting tubes, 1 ml of serum, 1 gm of meat, and 0.25 gm of hair were combined with 5 ml of 65% nitric acid and 3 ml of 30% hydrogen peroxide (Meligy, 2018; Waheed *et al* 2018). In 50 ml tubes, the samples were diluted to 50 mlwith MilliQ water (Millipore, Bedford, MA). After dilution and filtration with Whatman filter paper 1, the digested samples were analysed using atomic absorption spectrophotometry according to Meligy (2018) method.

Stock solutions and standard solution (1000 mg/L) of Cu, Cd, Fe, Zn, and Pb (Merck; Darmstadt, Germany) were used to create calibration curves. The average recovery rate ranged from 95 to 106%. Metal concentrations were calculated using standard curves for all metals studied. Wet weight (ww) basis was used to calculate all of the results.

Quality assurance and control

Measurement of IAEA142/TM from IAEA certified reference materials (muscle homogenate) was used to ensure the accuracy of the assay (Vienna, Austria). The certified samples' recovered concentrations were within 5% of the certified values. Triplicates of each sample were evaluated.

Estimated daily intake (EDI)

The following equation from the Human Health Evaluation Manual (US Environmental Protection Agency, 2010) was used to calculate the estimated daily intake (EDI) of the metals studied: EDI = Cm FIR / BW. Where, EDI is expressed in µg/kg/day; Cm is the metal concentration in the sample (measured in mg/kg wet weight); FIR is for Saudi Arabia's meat intake rate, which was assessed to be 146 grams per day; BW stands for Saudi adults and children body weight, which was assessed to be 70 kilograms for adults and 30 kilograms for children (Adam *et al,* 2014).

Health risk assessment

The non-cancer risk caused by the consumption of metal-contaminated edible tissues to the Saudi population (adults and children) was assessed using the guidelines set by the US Environmental Protection Agency (2010). The following equation was used to determine the hazard ratio (HR):

Hazard Ratio (HR) = EDI/RfD × 10×3.

Where EDI stands for estimated daily intake and RfD stands for recommended reference doses in mg/kg/ day (0.001 for Cd, 0.004 for Pb, 0.3 for Zn, 0.04 for Cu and 0.7 for Fe). To evaluate the risk of mixed metals, the hazard ratios (HRs) can be summed together to provide a hazard index (HI). The following equation was used to generate HI = $\sum$ HRi where i represent each metal. A HR and/or HI of ≥ 1 implies that there is a potential risk to human health, whereas a result of ≤ 1 shows that there is no risk of adverse health impacts.

Statistical analysis

SPSS (version 19) was utilised to conduct statistical analyses. To see if variables were normally distributed, the Kolmogorov-Smirnov normality test was used. One-way analysis of variance was applied to compare the means of the groups (ANOVA) when breed used as a factor. To examine the impact of breed on the analysed parameters, the Duncan multiple range test (Steel and Torrie, 1980) was used. The differences between genders were tested by independent t-test. The Pearson correlation coefficient was used to establish a link between variables and to confirm their significance. Statistical significance was determined at $p < 0.05$.

Results and Discussion

Zinc

Zinc is important for the activity of over three hundred enzymes that are responsible for digestion,

metabolism, nerve function, and other processes in man and animals (Zastrow and Pecoraro, 2014). The recorded data in table 1 declared that mean values of Zn ranged from 29.77 to 40.11, 11.58 to 13.36, 17.57 - 25.83, 14.84 - 17.93, 54.81- 90.45 mg kg^{-1} in liver, kidney, muscle, serum, and hair, respectively. The Zn values significantly varied between breeds ($P<0.05$). Furthermore values were arranged in descending manner, i.e. Hair> liver> muscle > kidney> serum. The maximum Zn concentration found in hair samples from different camel breeds was in accordance to the finding of Petukhov *et al* (2016) who examined muscle, tissues, and hair of cattle in western Siberia and found high level of Zn in hair. Relatively higher levels of Zn were reported in the liver compared with the muscle in previous studies as 34.4 mg kg^{-1} (Bakhiet *et al,* 2007), 43.67 mg kg^{-1} (Abdelrahman *et al,* 2013) and 70.625- 155.351 mg kg^{-1} (Asli *et al,* 2020) in livers. Comparable Zn values were obtained in camel meat 16.74 ± 0.73 - 40.17 ± 2.62 mg kg^{-1} (Alturiqi *et al,* 2012), mg kg^{-1} 10.88 ± 1.73 mg kg^{-1} (Chafik *et al,* 2014) and 23.254 to 49.991 mg kg^{-1} (Asli *et al,* 2020). Camel breed influences Zn accumulation and distribution in camel organs and muscle. Significant variations ($p < 0.05$) were found across breeds, which can be explained by each breed's environment being in a distinct geographical location with varying concentrations of metals in soil, forages, and water. Camel feeding, watering and even breathing resulted in Zn deposition in tissues and organs, which reflected the amount of environmental pollution. There was a strong relationship between soil and grasses, and the Zn content in forages was more than half that of soil (Yan *et al,* 2012). Regarding the effect of gender as shown in Table 2 all male collected samples had substantially greater ($P <0.05$) Zn levels than female samples, with no significance in hair. Nearly similar results were obtained in Iran where sex was a significant parameter affecting Zn level, i.e. male and female camels contained 39.128 and 34.616 mg kg^{-1} in meat, 120.743 and 102.947 mg kg^{-1} in liver, respectively (Asli *et al,* 2020). In addition, Faye *et al* (2008) detected significantly higher zinc level in male than female camels collected from Emirates. The correlation coefficient between Zn concentrations in organs and serum with breed and gender in table 3 revealed a strong positive correlation between muscle, liver, kidney, and serum. Moreover, a positive correlation was detected between serum and hair. The positive correlation attributed to Zn which can enter the body through the digestive tract from food or drink water or lungs after inhalation of Zn dust. Zn increases in blood rapidly after exposure. The negative correlation obtained for both of breed and gender may be attributed to accumulation of heavy metals in the animal body related to environmental factors such as air pollution, feeding, and available water sources.

Iron

Iron is a critical component for nearly all living creatures' development and survival (Valko *et al,* 2005). It is found in organisms like algae, enzymes like cytochromes and catalase, as well as oxygen-transporting proteins like myoglobin and hemoglobin (Vuori, 1995). Significant differences ($P<0.05$) were found between breeds according to their contents of iron between kidney and serum (Table 4). The iron variations in our findings are attributable to variances in the availability of iron in forages that are grown in the grazing areas. Comparable iron values were obtained 38.088- 77.364 mg kg^{-1} of muscle and 55.110- 101.927 mg kg^{-1} of liver samples in Iran (Asli *et al,* 2020). Meanwhile, higher iron values were found in liver 558.1 ± 266.4 mg kg^{-1} in Sudan (Tartour, 1969), 295.2 ± 21.6 mg kg^{-1} in Saudi Arabia (Al-Busadah, 2003) and 560.0 ± 38 mg kg^{-1} at eastern Sudan (Bakhiet *et al,* 2007). The variation in iron concentration in different studies may be due to the method of sample preparation and calculation according to wet weight or dry weight. In our study camels from all examined breeds showed a higher concentration of iron in the hair> liver > kidney> muscle> serum. But camel samples from Iran showed a higher concentration of iron in the liver > kidney > muscle > serum > hair (Badiei *et al,* 2006). The

Table 1. Effect of breed on zinc concentrations (PPM) in organs, muscle and camel serum.

	Wadha	Maghateer	Magaheem	SE	Minimum	Maximum	P value
Liver	40.111^{a}	29.771^{b}	30.461^{b}	1.1441	22.3	48.6	<0.001
Kidney	13.369^{a}	11.579^{b}	11.705^{b}	0.3414	7.4	16.1	0.054
Muscle	25.73^{b}	17.5727^{a}	25.8307^{b}	0.93315	10.08	31.78	<0.001
Serum	17.936	14.842	17.0427	0.68995	4.52	24.55	0.171
Hair	90.4513^{a}	54.8107^{b}	60.4587^{b}	2.7512	39.98	102.45	<0.001

Values with different superscript in a row different significantly ($P<0.05$).

iron content in all male camel samples studied was considerably greater ($P < 0.05$) than in female camel samples (Table 5). Iranian male and female camels had iron in muscle at levels of 60.10 and 59.31 mg kg^{-1}, respectively which was similar to our findings. In addition, adult male and female camel from Al-Najaf city, Iraq contained total serum iron as 84.043±1.74 and 79.985±2.83 µg/dl, respectively (Ghali and Al-Qayim, 2020). Of contrast, the serum iron of adult female camels in the Najdi breed in Central Saudi Arabia was greater than that of adult male camels (Hussein *et al,* 1997). The data in table 6 revealed a significant positive correlation between liver and kidney. Furthermore, significant strong positive correlation between muscle and serum was found. Torrance *et al* (1968) reported that muscle storage of iron can increase with increase iron in serum; breed and hair also showed significant strong positive correlation.

Copper

Although, copper accumulation in the inner organs is not the norm, adding copper to farm animal feed has been shown to result in increased copper levels in the liver (Franson *et al,* 2012). The results in table 6 declared that the lowest value for Cu was detected in serum sample of Magaheem breed 1.039± 0.05146 mg kg^{-1} meanwhile, highest value was 17.7873± 0.85 mg kg^{-1} detected in liver samples of Maghateer breed. The significant differences among breeds ($P< 0.05$) detected in kidney and hair that may attributed to the level of Cu in forages and rate of excretion and accumulation of Cu in kidney or hair of different breed. The Cu concentration

Table 2. Effect of gender on zinc concentration levels in organs, muscle and camel serum.

	gender	Mean	Std. Error	Minimum	Maximum	P
Liver	Male	39.133[a]	1.2809	29.2	48.6	<0.001
	Female	28.473[b]	1.0681	22.3	39.7	
Kidney	Male	13.942[a]	0.3206	10.5	16.1	<0.001
	Female	10.709[b]	0.3575	7.4	14.5	
Muscle	Male	27.29[a]	0.70011	21.67	31.78	<0.001
	Female	19.3296[b]	1.21027	10.08	29.65	
Serum	Male	20.5052[a]	0.50287	15.34	24.55	<0.001
	Female	13.1958[b]	0.65654	4.52	19.46	
Hair	Male	70.6305	4.54384	51.64	102.45	0.49
	Female	66.7738	3.33752	39.98	97.18	

Table 3. Correlations between zinc concentrations in organs and serum with breed and gender.

Zinc	Breed	Gender	Liver	Kidney	Muscle	Serum	Hair
Breed		.000	-.519**	-.300*	.007	-.080-	-.671**
Gender	.000		-.701**	-.712**	-.642**	-.797**	-.105-
Liver	-.519**	-.701**		.793**	.615**	.695**	.557**
Kidney	-.300*	-.712**	.793**		.580**	.691**	.358*
Muscle	.007	-.642**	.615**	.580**		.689**	.417**
Serum	-.080-	-.797**	.695**	.691**	.689**		.361*
Hair	-.671**	-.105-	.557**	.358*	.417**	.361*	

Values presented in the table are correlation coefficient
**. Correlation is significant at the 0.01 level *. Correlation is significant at the 0.05 level

Table 4. Effect of breed on Iron concentration (PPM) levels in organs, muscle and camel serum.

	Wadha	Maghateer	Magaheem	SE	Minimum	Maximum	P value
Liver	56.71	55.5	52.67	0.76	44.37	68.4	0.08
Kidney	50.73[b]	55.58[a]	55.7[a]	0.78	38.78	62.73	<0.01
Muscle	26.82	24.97	25.07	0.42	19.64	32.78	0.13
Serum	4.47[a]	3.23[b]	3.59[b]	0.16	1.65	6.23	<0.01
Hair	181.99	144.1	146.28	6.87	80.35	250.88	<0.05

in examined samples was arranged in descending manner as follows liver > muscle > hair > kidney > serum. Regarding Cu concentration, several values have been reported for camel livers in Sudan 6.5–125 mg kg^{-1} (Abu Damir *et al,* 1983), in Egypt 30–286 mg kg^{-1} (Khalifa *et al,* 1973), at Djibouti 19–88 mg kg^{-1} (Faye *et al,* 1992), in Saudi Arabia 265 ± 30 mg kg^{-1} (Al-Busadah, 2003), at the eastern region of Sudan 103 ± 12.3 mg kg^{-1} (Bakhiet *et al,* 2007), in Morocco 14 ± 6.12 mg kg^{-1} (Chafik *et al,* 2014) and in Iran 1.555–4.381mg kg^{-1} (Asli *et al,* 2020). The level of Cu reported as 1.10±0.24 and 1.43±0.14 mg kg^{-1} for muscle and kidney, respectively in Morocco (Chafik *et al,* 2014), 1.29±0.141 and 1.77±0.9 mg kg^{-1} for muscle and kidney, respectively in Egypt (Khalafalla *et al,* 2015), and 220- 2.940 mg kg^{-1} for meat in Iran (Asli *et al,* 2020). The effect of gender on the distribution of Cu showed no significant difference (P > 0.05) in between kidneys meanwhile, female livers contained significantly higher concentrations than the male liver. Male muscle, serum, and hair samples contained significantly higher Cu (P0.05) than female muscle, serum, and hair samples (Table 8). In previous reports sex had a significant effect on Cu concentration in camel samples (Rashed, 2002; Badiei *et al,* 2006). In other studies, sex had no significant effect on Cu in the tissues and serum of the camels (Chafik *et al,* 2014; Asli *et al,* 2020). The data in Table 9 revealed a positive significant correlation between liver and kidney. Furthermore, strong positive correlation was seen between muscle and serum; breed, and hair.

Lead

Lead and its compounds have accumulated in the environment, including air, water, and soil, because of human activities such as mining, manufacturing, and fossil fuel burning. Batteries, cosmetics, metal items such as bullets, solder and pipes are all made using lead (Jaishankar *et al,* 2014). Lead can negatively affect kidney function and haemopoiesis, as well as the gastrointestinal and nervous systems (Daniel *et al,* 1995). The lead residue was detected in all examined samples among different breeds except muscle samples of Maghateer and Magaheem breeds. The lowest detectable value obtained in muscle of Wadha breed 0.001 mg kg^{-1} and the highest value belongs to hair of Magaheem breed 1.979 ± 0.085 mg kg^{-1} and generally descending distribution of lead residue was hair > liver > kidney > serum > muscle (Table 10). The highest level of Pb in edible tissue obtained in liver come in parallel with Morshdy *et al* (2018) in Egypt and Bala *et al* (2018) in

Table 5. Effect of gender on iron concentrations in organs, muscle and camel serum.

	Gender	Mean	Std. Error Mean	Minimum	Maximum	P
Liver	Male	58.622[a]	0.795	52.360	68.400	<0.001
	Female	51.761[b]	0.815	44.370	60.340	
Kidney	Male	57.105[a]	0.762	49.670	62.730	<0.001
	Female	51.285[b]	1.025	38.780	58.990	
Muscle	Male	26.906[a]	0.551	22.770	32.780	<0.01
	Female	24.493[b]	0.539	19.640	29.770	
Serum	Male	4.473[a]	0.186	3.220	6.230	<0.001
	Female	3.149[b]	0.185	1.650	4.670	
Hair	Male	185.941[a]	8.833	110.360	250.880	<0.001
	Female	132.538[b]	7.232	80.350	210.340	

Table 6. Correlations between iron concentrations in organs and serum with breed and gender.

Iron	Breed	Gender	Liver	Kidney	Muscle	Serum	Hair
Breed		.000	.082	0.161	-.021-	-.021-	0.428**
Gender	.000		.284	-.207-	-.399**	-.399**	-.453**
Liver	.082	.284		.375*	-.375*	-.375*	-.080-
Kidney	.161	-.207-	.375*		-.112-	-.112-	.217
Muscle	-.021-	-.399**	-.375*	-.112-		1.000**	.291
Serum	-.021-	-.399**	-.375*	-.112-	1.000**		.291
Hair	.428**	-.453**	-.080-	.217	.291	.291	

Values presented in the table are correlation coefficient based on Pearson Correlation coefficients.
**. Correlation is significant at the 0.01 level *. Correlation is significant at the 0.05 level.

Nigeria. Comparable to our finding the lead was not detected in camel meat from Iran (Asli *et al,* 2020) meanwhile, lead residues were detected in camel muscle from Egypt as 1.402±0.52 mg kg^{-1} (Khalafalla *et al,* 2015) and in camel meat from Al-Ahsa Abattoir, Saudi Arabia as 0.00730 ± 0.0012 mg kg^{-1} (Meligy *et al,* 2019). The examined camel liver samples in our study contained (0.142- 0.204 mg kg^{-1}) which were relatively lower than 3.4 ± 0.31 mg kg^{-1} in camel livers from Egypt (Khalafalla *et al,* 2015), (0.093–1.563 mg kg^{-1}) in Iranian camel livers (Asli *et al,* 2020). The residual level of lead in the kidney ranged from 0.123 to 0.323 mg kg^{-1} meanwhile, higher lead values in kidney tissues was obtained 1.41±0.23 mg kg^{-1} in Egypt (Khalafalla *et al,* 2015), 5.05-11.88 mg kg^{-1} in Saudi Arabia (El-Ghareeb *et al,* 2019). The hair samples contained lead residues from 0.612 to 2.615 mg kg^{-1} in our study meanwhile, higher values were obtained in camel hair samples 4± 0.51 to 13±4.32 mg kg^{-1} from different locations in Egypt (Rashed and Soltan, 2005). The variation in the level of lead contamination may be attributed to differences in accumulation rate in the environment, such as air, water, and soil due to human activities like manufacturing, mining, and fossil fuel burning. All the examined camel muscle and offal in our study below the maximum permissible limit of Pb (0.1 and 0.5 mg/kg ww for muscle and offal, respectively) established by the European Commission (2006). There is no significant difference in lead residue (P> 0.05) related to gender among examined samples (Table 11) indicating that equal exposure to lead sources between males and females. The correlation between lead concentration in Table 12 revealed a positive correlation between liver and breed while the strong positive correlation between breed, serum, and hair.

Table 7. Effect of breed on copper concentrations in organs, muscle and camel serum.

	Wadha	Maghateer	Magaheem	SE	Minimum	Maximum	P value
Liver	13.6053	17.7873	14.7349	0.85	7.59	30.44	0.114
Kidney	1.26227^{b}	2.03527^{a}	1.46527^{b}	0.077759	0.735	2.746	0.000
Muscle	2.1133	2.2213	2.078	0.10291	1.04	3.57	0.845
Serum	1.0567	1.1107	1.039	0.05146	0.52	1.78	0.845
Hair	2.2427^{b}	3.4974^{ab}	3.8559^{a}	0.23199	1.02	6.78	0.009

Table 8. Effect of gender on copper concentrations in organs, muscle and camel serum.

	Gender	Mean	Std. Error Mean	Minimum	Maximum	P
Liver	Male	13.662^{b}	1.397	52.36	68.4	0.05
	Female	16.875^{a}	0.946	44.37	60.34	
Kidney	Male	1.701	0.137	49.67	62.73	0.19
	Female	1.487	0.079	38.78	58.99	
Muscle	Male	2.428^{a}	0.11	22.77	32.78	0.007
	Female	1.882^{b}	0.15	19.64	29.77	
Serum	Male	1.214^{a}	0.055	3.22	6.23	0.006
	Female	0.941^{b}	0.075	1.65	4.67	
Hair	Male	3.944^{a}	0.328	110.36	250.88	0.002
	Female	2.546^{b}	0.267	80.35	210.34	

Table 9. Correlations between copper concentrations in organs and serum with breed and gender.

Copper	Breed	Gender	Liver	Kidney	Muscle	Serum	Hair
Breed		.000	.082	.161	-.021-	-.021-	.428**
Gender	.000		.284	-.207-	-.399**	-.399**	-.453**
Liver	.082	.284		.375*	-.375*	-.375*	-.080-
Kidney	.161	-.207-	.375*		-.112-	-.112-	.217
Muscle	-.021-	-.399**	-.375*	-.112-		1.000**	.291
Serum	-.021-	-.399**	-.375*	-.112-	1.000**		.291
Hair	.428**	-.453**	-.080-	.217	.291	.291	

*. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Cadmium

Humans are most exposed to Cd by inhalation and ingestion and can develop acute and chronic intoxications (Jaishankar *et al,* 2014). It can also cause kidney dysfunction, increases in blood pressure, hepatocellular and pulmonary damage (Daniel *et al,* 1995). The results in table 13 showed that Cd values ranged from 0.0001 mg kg^{-1} in muscle of Maghateer breed to 4.5113 mg kg^{-1} in hair of Wadha breed. There were no significant differences between breeds in all examined samples except significant lower value in hair samples of Maghateer breed than other breeds. Comparable Cd values were obtained in Iranian camel muscle 0.006-0.012 mg kg^{-1} (Asli *et al,* 2020). On the other hand, the residual level of Cd in liver, kidney and muscle in this study was greatly lower than 0.46 ± 0.09, 0.85 ± 0.26 and 0.2 ± 0.03 mg kg^{-1}, respectively observed in Egypt (Khalafalla *et al,* 2015), 1.95, 1.82 and 0.15 mg kg^{-1}, respectively in Saudi Arabia (El-Ghareeb *et al,* 2019). The Cd residues in examined meat and offal in this study were within the maximum permissible limits (MPLs) of Cd in the meat and organ (0.5 to 1.0 mg/kg ww, respectively) established by the European Commission (2006). The hair samples in this study contained the highest Cd residue level ranged from 2.0937 to 4.5113 mg kg^{-1}. Nearly similar Cd residues in camel hair obtained 0.25 ± 0.09 to 5.75 ± 0.87 mg kg^{-1} in Egypt (Rashed and Soltan, 2005). In addition, Medvedev (1999) reported lower Cd residue in camel hair 0.25 mg kg^{-1}. Animal hair, with its unique capacity to preserve the picture of environment imprinted over time, is becoming more important than other tissues in assessing heavy metal concentrations in the environment in which animals were reared. According to the researchers, animals eliminate pollutants by sequestering them in their hair, and molting is the primary method of heavy metal excretion (Rose and Parker, 1982; Braune and Gaskin, 1987; Honda *et al,* 1987). According to data in table 14 males had significantly

Table 10. Effect of breed on lead concentration levels in organs, muscle and camel serum.

	Wadha	Maghateer	Magaheem	SE	Minimum	Maximum	P value
Liver	0.142^{b}	0.204^{a}	0.179^{ab}	0.008	0.088	0.287	0.002
Kidney	0.240^{a}	0.190^{b}	0.171^{b}	0.007	0.123	0.323	0.000
Muscle	0.001	0.000	0.000	0.000	0.000	0.005	0.102
Serum	0.004^{b}	0.003^{b}	0.040^{a}	0.003	0.000	0.081	0.000
Hair	1.052^{b}	0.892^{b}	1.979^{a}	0.085	0.612	2.615	0.000

Table 11. Effect of Gender on lead concentration levels in organs, muscle and camel serum.

	Gender	Mean	Std. Error Mean	Minimum	Maximum	P
Liver	Male	0.177	0.009	0.111	0.268	0.82
	Female	0.173	0.012	0.088	0.287	
Kidney	Male	0.208	0.011	0.124	0.323	0.32
	Female	0.194	0.009	0.123	0.290	
Muscle	Male	0.001	0.000	0.000	0.005	0.21
	Female	0.000	0.000	0.000	0.002	
Serum	Male	0.020	0.005	0.000	0.076	0.22
	Female	0.012	0.004	0.000	0.081	
Hair	Male	1.486^{b}	0.136	0.790	2.615	0.04
	Female	1.152^{a}	0.099	0.612	1.955	

Table 12. Correlations between Lead concentrations in organs and serum with breed and gender.

Lead	Breed	Gender	Liver	Kidney	Muscle	Serum	Hair
Breed		0.000	.302*	-.607**	-0.289-	0.657**	0.669**
Gender	0.000		-0.035-	-.151-	-0.207-	-.189-	-0.295*
Liver	0.302*	-.035-		-.125-	-.006-	-.166-	-0.092-
Kidney	-.607**	-.151-	-.125-		0.198	-.355*	-0.189-
Muscle	-0.289-	-0.207-	-0.006-	0.198		-0.115-	-0.034-
Serum	.657**	-.189-	-.166-	-.355*	-.115-		0.744**
Hair	0.669**	-0.295*	-.092-	-0.189-	-.034-	0.744**	

higher Cd values (P< 0.05) in liver and serum than females, which may attributed to higher feed intake than females. On the contrary, females contained higher Cd in hair samples may be due to long life in breeding and reproduction then introduced to slaughter. Liang *et al* (2017) reported that heavy metal concentrations varied according to age groups, and higher concentrations for Cd appeared in female hair. The data in table 15 revealed a significant positive correlation between muscle and liver in addition to a strong significant positive correlation muscle, serum and hair, which attributed to the action of hair as a biological matrix is it contains information about metabolic pools of toxic elements in animals (Miroshnikov *et al*, 2019).

Health risk assessment

The health risk assessment due to consumption of camel meat from different breeds in Saudi Arabia *via* estimated daily intake (EDI) in comparison with tolerable daily intake (TDI). The calculated data in Table 16 revealed that EDI of all examined metals from all breeds below the TDI established by FAO/WHO (2010). The EDI of toxic metal as Pb and Cd was lower than previous report in Nigeria from cattle meat (Ihedioha and Okoye, 2013), in Saudi Arabia from camel meat (El-Ghareeb *et al*, 2019) and in Iran from cattle meat (Zeinali *et al*, 2019). The non-carcinogenic hazard ratios (HRs) and hazard indices (HIs) were assessed in this study in Table 16. HIs due to consumption of Wadha breed slightly higher than Maghateer or Magaheem but all HR and HIs values were far below 1, which proved no potential exposure to risk due to consumption of camel meat from different breeds. Our results in the same line with Orellana *et al* (2021) who find that no undesirable health risk for adults and children combined with the consumption of Alpaca meat in Huancavelica, Peru.

Table 13. Effect of breed on cadmium concentration levels in organs, muscle and camel serum.

	Wadha	Maghateer	Magaheem	SE	Minimum	Maximum	P value
Liver	0.0039	0.0127	0.0091	0.0012	Not detected	0.0345	0.009
Kidney	0.0101	0.0072	0.0049	0.0014	0.0005	0.0657	0.308
Muscle	0.0009	0.0001	0.0002	0.0002	0.0000	0.0050	0.066
Serum	0.0153	0.0195	0.0175	0.0010	0.0004	0.0357	0.231
Hair	4.5113[a]	2.0937[b]	4.1480[a]	0.2749	0.8700	8.2400	<0.0001

Table 14. Effect of Gender on cadmium concentration levels in organs, muscle and camel serum.

	Gender	Mean	Std. Error	Minimum	Maximum	P
Liver	Male	0.011419[a]	0.002024	Not detected	0.0345	0.03
	Female	0.006074[b]	0.001292	0.00065	0.02031	
Kidney	Male	0.00985	0.002813	0.00347	0.0657	0.09
	Female	0.005234	0.000453	0.00046	0.00954	
Muscle	Male	0.00017	0.000042	0.0001	0.0008	0.14
	Female	0.00059	0.000276	0.0001	0.005	
Serum	Male	0.021172[a]	0.001278	0.01467	0.0357	<0.001
	Female	0.014155[b]	0.001184	0.00039	0.02111	
Hair	Male	2.5419[b]	0.24543	0.87	4.56	<0.001
	Female	4.4965[a]	0.38433	2.01	8.24	

Table 15. Correlations between cadmium concentrations in organs and serum with breed and gender.

Cadmium	Breed	Gender	Liver	Kidney	Muscle	Serum	Hair
Breed		**.000**	**-.325***	**.391****	**-.256-**	**-.334***	**-.320***
Gender	.000		**-.674****	**-.561****	**-.430****	**-.609****	**-.584****
Liver	-.325*	-.674**		**.257**	**.330***	**.627****	**.543****
Kidney	.391**	-.561**	.257		**.247**	**.149**	**.165**
Muscle	-.256-	-.430**	.330*	.247		**.578****	**.463****
Serum	-.334*	-.609**	.627**	.149	.578**		**.765****
Hair	-.320*	-.584**	.543**	.165	.463**	.765**	

Table 16. Estimated daily intake (µg/kg/day) and risk assessment (HR and HI) due to ingestion of camel meat from different breeds.

		Zn	Iron	Cu	Pb	Cd	HIs
Wadha	EDI (Adult)	53.67	55.94	4.41	0.00208	0.00188	
	EDI (child)	125.22	130.52	10.28	0.00486	0.00438	
Maghateer	EDI (Adult)	36.65	52.08	4.63	0	0.00021	
	EDI (child)	85.52	121.52	10.81	0	0.00048	
Magaheem	EDI (Adult)	53.88	52.29	4.33	0	0.00042	
	EDI (child)	125.71	122.01	10.11	0	0.00097	
	TDI	1000	800	500	3.57	1	
Wadha	HR (Adult)	0.18	0.08	0.11	0.00052	0.00188	0.371
	HR (child)	0.42	0.19	0.26	0.00121	0.00438	0.867
Maghateer	HR (Adult)	0.12	0.07	0.12	0	0.00021	0.313
	HR (child)	0.29	0.17	0.27	0	0.00048	0.729
Magaheem	HR (Adult)	0.18	0.07	0.11	0	0.00042	0.363
	HR (child)	0.42	0.17	0.25	0	0.00097	0.847

TDI: Tolerable daily intake according to FAO/WHO (2010)

However, much care should be taken as exposure to heavy metals also from vegetables, water and air which may increase the HIs especially in children. The obtained values for HR and HIs were found lower than those seen in Ghana (Bortey-Sam *et al*, 2015), Egypt (Darwish *et al*, 2015), Iran (Zeinali *et al*, 2019), and in Saudi Arabia (El-Ghareeb *et al*, 2019).

It was concluded that heavy metals distributed among camel samples of different breeds and toxic metal Pb and Cd in meat and offal were below the international maximum permissible limit. The correlation between samples reflected the role of hair as a good tool for identification of heavy metal pollution. In addition, no potential health hazards among camel meat consumers in Saudi Arabia especially, adults were seen.

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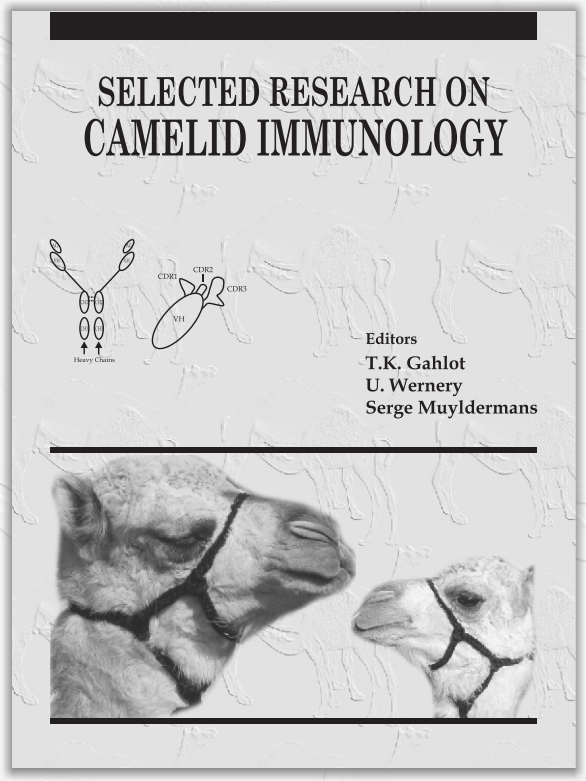
In 1989 a group of biologists led by Raymond Hamers at the Free University Brussels investigated the immune system of dromedaries. This discovery was published in Nature in 1993. Based on their structure, these peculiar camelid antibodies have been named Heavy Chain Antibodies (HCAb), as they are composed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



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