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CAMEL FORENSIC MEDICINE- A CHALLENGING SPECIALISATION

Camel forensic medicine is an emerging branch of veterinary science that applies forensic principles to legal cases involving camels. Its importance lies in determining the cause of injury or death in camels in medicolegal contexts, investigating animal cruelty, and providing expert analysis in cases such as insurance claims, ownership disputes, and vehicle collisions involving camels. The primary institute specialising in camel forensic medicine in the Arab world is the Camel Forensic Laboratory located in Dubai, United Arab Emirates. It is part of the Central Veterinary Research Laboratory (CVRL) and provides testing and research facilities to the UAE and neighboring countries. Other notable camel veterinary and research centres that may handle forensic pathology as part of their broader work include Dubai Camel Hospital, Advanced Scientific Group (Abu Dhabi), ADAFSA Collaborating Centre for Camel Diseases (Abu Dhabi), Camel Research Centre- Dept of President's Affairs (Abu Dhabi): which in an impressive facility for camel and falcon research. In Saudi Arabia, the Salam Veterinary Hospital (Beda, Buraydah) is known as the world's largest camel hospital, this state-of-the-art facility combines modern medicine with advanced research, including extensive laboratories equipped for over 160 types of analyses essential for diagnosing diseases. Tharb Camel Hospital (Al Uwaynah) of Qatar is a specialised hospital offering a range of medicines and treatments for camels with all necessary facilities and staff. These institutions focus on various aspects of camel health, disease, genetics, and welfare, often collaborating with international bodies like the World Organisation for Animal Health (WOAH) through initiatives like CAMENET (Camel Middle East Network) for disease surveillance and pathology.

Forensic pathology in camels helps veterinarians and law enforcement determine if injuries or death were accidental, natural, or a result of malicious intent or neglect. Veterinary forensics provides the necessary tools and evidence to investigate and prosecute cases of abuse or neglect, ensuring animal welfare protection. Identification of remains and origin is done through the techniques such as DNA analysis (including mitochondrial DNA sequencing) which are used to determine the species of origin of animal remains or products. Forensic studies, such as the detailed anatomical and radiological descriptions of camel skulls, help veterinarians differentiate between fractures caused by trauma and natural anatomical features like sutures, which is critical for accurate diagnosis and legal documentation. Forensic investigations help in tracing disease outbreaks such as Middle East Respiratory Syndrome (MERS-CoV) and understanding transmission dynamics, which is crucial for public health and safety. Forensic veterinarians provide crucial evidence and expert testimony in legal cases (e.g., insurance claims, criminal prosecutions), requiring standardised methodologies for sample handling, documentation, and analysis to ensure the evidence is admissible in court.

There is need for standardised research in camel forensic medicine that can also lead to a better scientific understanding of camel biology, disease resistance, and unique physiological adaptations, which can have broader applications in general veterinary medicine and potentially human health (e.g., nanobodies research). In essence, camel forensic medicine provides the robust scientific and legal framework to address complex medicolegal issues involving camels, an animal of significant cultural, economic, and medicinal importance in many parts of the world.

Current issue has many good manuscripts on diverse topics which will enrich the knowledge of dromedary and Bactrain camel science to the readers. These include the role of acute-phase proteins as biomarkers for health and disease in camels: a comprehensive review, gross and microscopic hepatic lesions of dromedary camels slaughtered in eastern province of Kingdom of Saudi Arabia, ncRNAs regulation of salt and drought resistances in camels, my journey to camel science by Rolf Karl Schuster, Saritha Sivakumar, Abdelmalik Khalafalla and Lulu Skidmore, fracture management

in the racing camel, rift valley fever – a neglected pathogenic virus of camelidae, seroepidemiological studies for the detection of antibodies of six infectious diseases in Kenyan dromedary camels, application of bactrian camel-derived nanobodies in the detection of foodborne pathogens, joint injections in camels: a review, haematological variations before and affter blood transfusion in Arabian racing camels, electrocardiographic measurements in the camel, assessment of camel feed resources: woody species stand structure, species richness, and diversity in Tsabong Ecotourism Camel Park, south-western Botswana, target-centric approaches for camel milk adulteration detection: a review of protein-, metabolite- and gene-based analytical strategies and first phenotypic characterisation of local dromedary camel ecotype in el oued region, southeast Algeria.

I congratulate the entire team of Editorial board of JCPR whose services and contributions since last 32 years has led to international recognition to this journal in form of Gourmand Award which was received by me at Riyadh, Saudi Arabia on 30th November 2025. I wish all the members of the editorial board and our esteemed readers a happy Christmas and New Year 2026.

(Dr. Tarun Kumar Gahlot)
Editor

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ELECTROCARDIOGRAPHIC MEASUREMENTS IN THE CAMEL (*Camelus dromedarius*)

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ABSTRACT

This study was aimed to establish reference electrocardiographic parameters in healthy adult female camels using standardised acquisition and analysis protocols. Sixteen camels aged 9.5 ± 0.2 years and weighing 460.8 ± 19.9 kg were examined. ECGs were recorded in sternal recumbency employing a modified base–apex configuration with adhesive electrodes. Data acquisition was performed with a PowerLab system at 2 kHz, followed by high-order, linear, low-pass filtering and waveform annotation in MATLAB. Thirty sequential beats per animal were analysed for P wave duration, PR segment, PR interval, QRS duration, ST segment, QT interval, T wave duration, RR interval, heart rate (HR), heart rate variability (HRV) and T/QRS amplitude ratio. After exclusion of records with excessive noise and arrhythmias, analysis revealed a mean P wave duration of 90ms, PR interval of 221ms, QRS duration of 79ms, ST segment of 185ms, QT interval of 382ms and T wave duration of 118ms, RR interval 987ms. The average HR was 61bpm, with HRV of 49ms and a T/QRS amplitude ratio of 26%. Compared to earlier studies, these findings confirm species-specific features such as prolonged atrioventricular conduction and low-amplitude QRS complexes. Methodologically, adhesive electrodes combined with sternal recumbency provided improved signal quality and minimised motion artifacts over restraint in a standing position. This work contributes updated normative data for camel ECG interpretation and underscores the feasibility of using modern acquisition systems for consistent measurements. Establishing reliable ECG benchmarks is critical for diagnosing arrhythmias and conduction abnormalities in camels, with implications for health monitoring in racing, dairy and working animals. Further studies across ages, breeds, gender and physiological states are recommended to expand reference ranges.

Key words: Camel (*Camelus dromedarius*), electrocardiogram, heart

The electrocardiogram (ECG) is a valuable non-invasive tool for evaluating cardiac electrical activity, diagnosing arrhythmias and assessing conduction abnormalities in animals. While extensively utilised in domestic species, studies on the ECG characteristics of the dromedary camel remain relatively scarce (Geddes, 2002). Given the increasing interest in camel physiology, driven by the animal's growing involvement in racing, dairy production and cultural heritage, there is a pressing need to establish robust electrocardiographic reference values and methods tailored to the unique anatomy and physiology of this species. Early investigations into the camel ECG reported marked species-specific features, including sinus bradycardia, long atrioventricular conduction times (PR intervals) and low-amplitude QRS complexes compared to humans. Braun *et al* (1958) recorded an ECG in a male 265 kg camel and reported heart rates ranging between 24 and 30 bpm. P waves were well defined with maximum width of 0.26 sec and PR interval ranging from 0.24 to 0.26 sec and maximal duration of the QRS complex was 0.09 sec. The QT interval ranged from 0.54 to 0.60 sec (Braun *et al,* 1958). Geddes *et al* (1973) recorded an ECG in an elderly male 507 kg camel under anaesthesia. Heart rate was 77 bpm, P wave duration of 0.1 sec, a long atrioventricular conduction time, with a PR interval of 0.26 sec, QRS duration of 0.09 sec, QT duration of 0.42 sec and T-wave duration of 0,12 sec and an unusual QRS axis (+250°), highlighting differences in impulse conduction likely attributable to anatomical and electrophysiological adaptations in camels (Geddes *et al,* 1973). Previous studies in camel have demonstrated the effects of intravenous administration of furosemide on clinical variables including electrocardiographic indices in young camel

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calves (Samimi and Sanjarinejad, 2021). A recent study by Babiker *et al* (2024) documented a rare case of traumatic reticulopericarditis in camel (Babiker *et al,* 2024). More recent studies have focused on refining ECG acquisition techniques, with the base–apex lead system emerging as the preferred configuration due to its simplicity, stability and reduced artifact susceptibility. Pourjafar *et al* (2011b) demonstrated the reliability of the base–apex system across various age groups, revealing age-dependent variations in ECG parameters while confirming consistent features such as a prolonged PR interval and a high prevalence of sinus arrhythmias. Camels were studied in the age range 6 months to 18 years of age. Heart rates ranged from 48 in 16-18 year compared to 89 bpm in less than 6 month old camels. The long atrioventricular node conduction time (the PR interval) was also an important finding in this study. The PR interval was 0.20-0.21 and 0.21-0.26 sec. in calves and adults, respectively. The RT segment was 0.24-0.26 and 0.19-0.30 sec in calves and adults, respectively (Pourjafar *et al,* 2011b). Clinical case reports have also expanded our understanding of pathological ECG patterns in camels. Atrial fibrillation, although rarely documented in this species, has been identified and characterised electrocardiographically by the absence of P waves and the presence of multiple fibrillatory waves with irregular RR intervals and tachycardia in a 5-year old male camel (Pourjafar *et al,* 2011a). The integration of modern technology, including wearable fitness trackers such as Equimetre™, has further enhanced camel ECG monitoring. Comparative studies have shown that these devices can produce clinically acceptable ECG recordings in both healthy and diseased camels, potentially offering practical solutions for field-based assessments in remote desert environments (Al Khamis *et al,* 2023). Despite these advances, comprehensive ECG studies in the camel are few and far between. This research was aimed to provide further detailed electrocardiographic features of the ECG in female camels, aged 9.5±0.2 years and weighing 460.8±19.9 kg, making use of modern methodologies in terms of acquisition hardware and analysis software, thereby contributing to a deeper understanding of camel cardiac electrophysiology and establishing a foundation for improved clinical practice and research in camelid medicine.

Materials and Methods

Animals

Apparently healthy female camels, aged 9.5±0.2 years and weighing 460.8±19.9 kg, accommodated at the Camel Research Centre, Al Ain were used in this study. Ethical approval for the project was obtained from the UAE University Animal Ethics Committee. Animals were weighed before measurement of the ECG.

Electrocardiographic recording

Camels were seated in a sternal recumbency position as shown in Fig 1. Hair was removed from electrode locations with an electronic razor followed by a hand razor. The area was cleaned with ethanol. A small bead of ECG Gel (Konix) was placed on each of three disposable ECG adhesive button electrode pads (Sino-K, X0024ZRZRN). The electrodes were then attached to the skin. Electrodes were placed in a modified apex-base configuration as shown in Fig 1. The negative electrode was placed on the right side of the neck, in the jugular groove, about one-third the distance from the mandible to the thoracic inlet (Fig 1A). The positive electrode was placed on the left side of the thorax, just caudal to the olecranon, the point of the elbow and slightly above the cardiac apex (Fig 1B). The ground electrode was placed on the withers, the highest point of the shoulders (Fig 1C).

Electrical recording

The electrodes were connected *via* cables to a PowerLab 26T (ADInstruments, ML856). The PowerLab was connected to a laptop computer. ECG data was acquired at a sampling rate of 2k/sec with LabChart 7 software (v7.3.8, ADInstruments). Data acquisition was continued for a period of 3 minutes.

Data analysis

The LabChart datafiles were saved in MATLAB format (R2024a) and filtered using a high order, FIR low pass filter with a 150Hz cutoff frequency in order to reduce baseline noise. After visual inspection, the best 30 sequential beats were selected for ECG parameter evaluation. Specifically, for each beat, the following was identified: P wave start and end, QRS complex and T wave start, end and amplitude. From the identified parameters, the P Wave duration, PR Segment, PR Interval, QRS Complex, ST Segment, QT Interval, T Wave duration, RR Interval, T/QRS Ratio, Heart Rate (HR) and Heart Rate Variability (HRV) values were determined. The HR was determined from the RR Interval and the short term HRV was determined from the standard deviation of the normal RR Interval (SDNN).

Statistical analysis

The ECG of 16 camels were recorded and analysed. Of the 16, excessive baseline noise

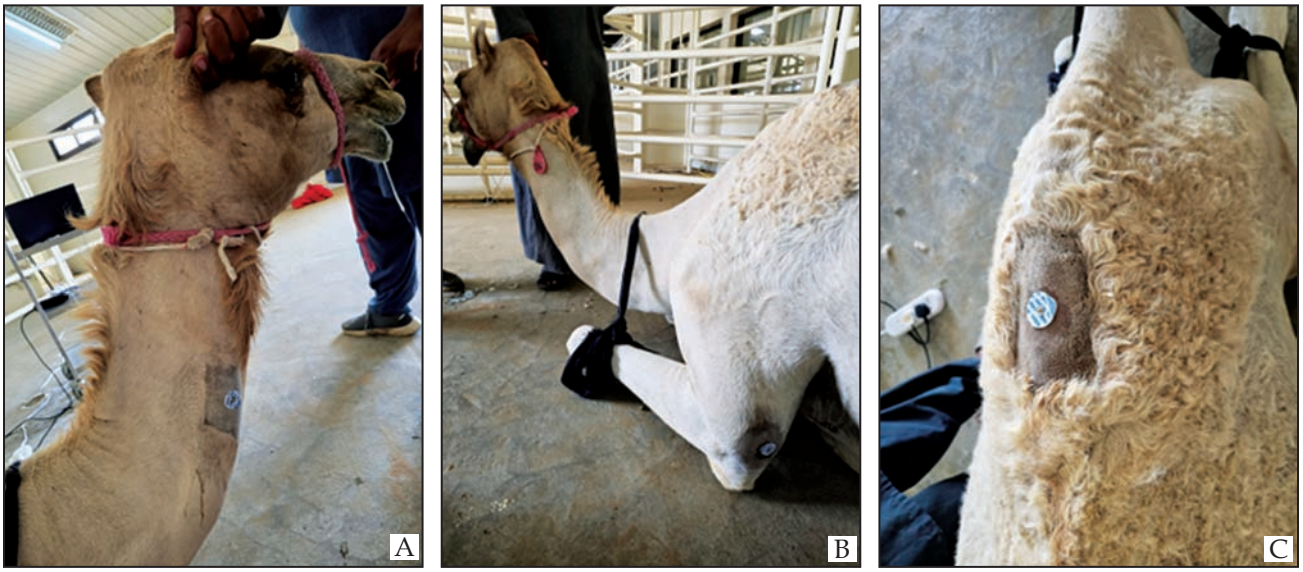

Fig 1. Photographs showing positioning of electrodes in a camel seated in the sternal recumbent position. The negative electrode was placed on the right side of the neck, in the jugular groove, about one-third the distance from the mandible to the thoracic inlet (A). The positive electrode was placed on the left side of the thorax, just caudal to the olecranon, the point of the elbow and slightly above the cardiac apex (B). The ground electrode was placed on the withers, the highest point of the shoulders (C).

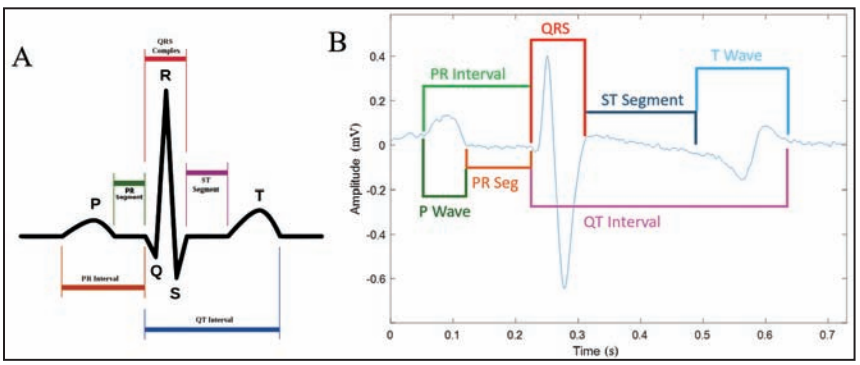

Fig 2. ECG showing P wave, QRS complex, T wave durations and PR interval, PR segment, ST segment and QT interval (A) taken from: https://en.wikipedia.org/wiki/Electrocardiography. A typical camel ECG displaying similar detection locations (B).

of one camel prevented identification of interval parameters beyond the QRS complex and was eliminated from the summative results. Four of the 16 camels (25%) displayed bradyarrhythmia and were similarly eliminated. An additional three of the 16 camels (19%) presented tachycardia and were also eliminated from the summative results. Sample camel bradyarrhythmia and tachycardia are displayed in Fig 4. For each of the identified parameters, the measured maximum, minimum and central tendencies were estimated using sample mean ± the standard error and sample median ± the interquartile range (IQR), as displayed in table 1.

Results

Of the 16 camels in the study, 44% presented arrhythmia associated with bradyarrhythmia (irregular diastasis duration) or tachycardia with a minimal diastasis duration, defined as the time between the end of the T wave and start of the P wave for the next cardiac cycle. With the elimination of an additional camel due excessive baseline noise, the summative results are based on 50% (n=8) of camels in the study, as displayed in table 1. It was noted that inclusion of the ECG parameters from camels with arrhythmia had little effect on the central tendency values beyond the RR Interval, HR and HRV parameters. Definitions of the various ECG waves, segments and intervals are shown in Fig 2A and the measurements made on a typical ECG record are displayed in Fig 2B. Typical ECG recordings are shown in Fig 3. The left panels show 10 second recordings and the right panels show 1 second expanded time scale recordings. Fig 3A shows an ECG record with a positive P wave, negative QRS and positive T wave. Fig 3B shows an ECG record with a positive P wave, biphasic QRS and a positive T wave. Fig 3C shows an ECG record with a positive P wave, biphasic QRS and negative T wave. Examples of arrhythmias are shown in Fig 4. A bradyarrhythmia is displayed in Fig 4A and a tachyarrhythmia is displayed in Fig 4B.

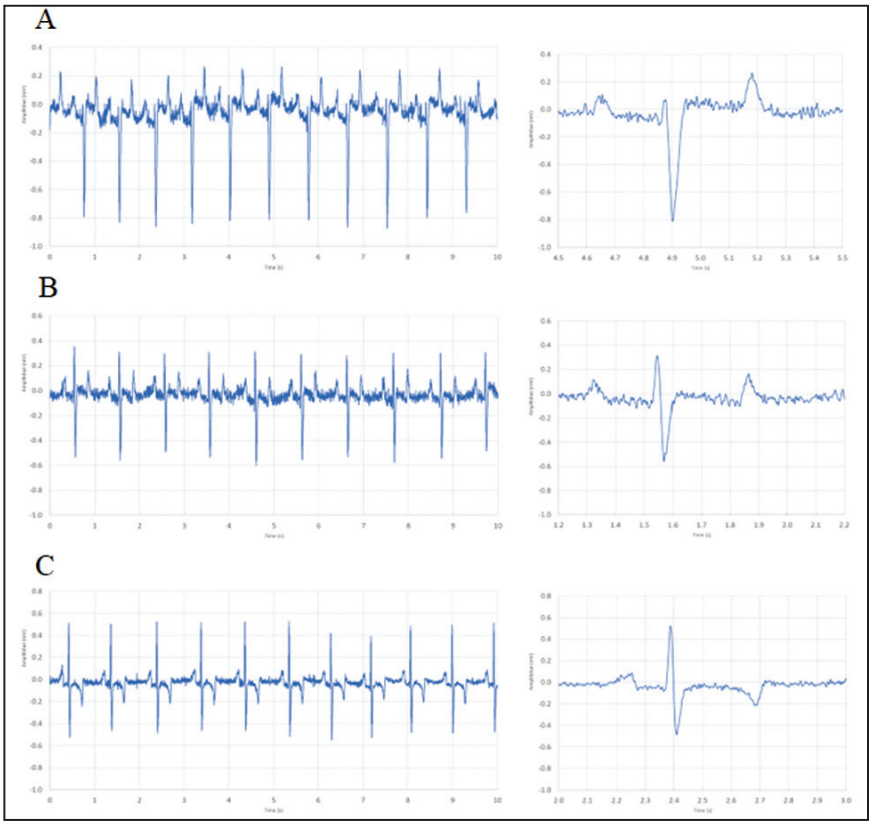

Fig 3. Electrocardiographic recordings of the various orientations of P, QRS and T waves. The left panels show a 10 sec strip, the right panels show an expanded scale 1 sec strip. P wave positive, QRS complex negative, T wave positive (A), P wave positive, QRS complex biphasic, T wave positive (B) P wave positive, QRS complex biphasic, T wave negative (C).

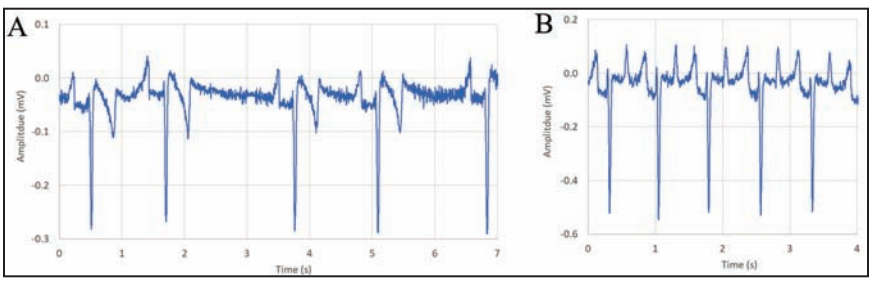

Fig 4. Sample camel arrhythmia: bradyarrhythmia (A) and tachyarrhythmia (B).

Discussion

Measurement of the ECG in camels is not easy. Initially, attempts were made to measure the ECG in standing camels restrained in a crusher. However, the camels were still able to move and the signals were often noisy. The camels were then allowed to sit in the sternal recumbency position (Fig 1A–C). The camels seemed more relaxed in this position and so we adopted the seated approach for this study. Two types of electrodes were used. The first was a crocodile clip the second a commercially available adhesive button electrode. The advantage of the former was that the clip could be simply attached with no preparation in terms of shaving the hair. The second approach and the one that was adopted in this study, required careful shaving of the hair before attachment of the adhesive electrodes to the skin (Fig 1A–C). The latter arrangement seemed to generate better ECG recordings with less baseline noise and was employed in this study. The mean age of the female camels without arrhythmia (n=8) was 9.5±0.4 years and the body weight was 495.6±18.5 kg. The ECG was recorded in a modified base-apex configuration with the electrodes placed as shown in Fig 1A-C.

A variety of ECG parameters were recorded. The P wave duration is the time it takes for the electrical impulse to travel through the atria, initiating contraction. The P wave duration was 90±6ms (n=8). Previous studies in camel using base-apex configuration have reported P wave durations of 60-90ms in camels ranging in age from <6 months to 16–18 years of age (Pourjafar *et al,* 2011b). For comparison in cattle a P wave duration of 50–120ms has been reported (Devadevi *et al,* 2022).

The PR segment represents the depolarisation of the atrioventricular (AV) node. The PR segment was 130±9ms (n=8). Previous studies in camel have reported 100ms (range 80–150ms) (Al Khamis *et al,* 2023). The PR interval represents the time it takes for an electrical impulse to travel from the sinoatrial (SA) node through the atria and AV node to the ventricles. The PR interval was 221±14ms (n=8). Previous studies in camel have reported 200–260ms ranging in age from <6 months to 16-18 years of age (Pourjafar *et al,* 2011b) and 200ms (range 160–260ms) (Al Khamis *et al,* 2023). For comparison in cattle a PR duration of 120–260ms has been reported (Devadevi *et al,* 2022).

The QRS complex represents ventricular depolarisation, which leads to their contraction. The

QRS complex duration was 79±3ms (n=8). Previous studies in camel have reported 110ms (range 100–190ms) (Al Khamis *et al,* 2023) and for comparison in cattle 40–100ms (Devadevi *et al,* 2022).

The ST segment represents the period where the ventricles are contracting and actively expelling blood. The ST segment duration was 185±6ms (n=8). Previous studies in camel have reported 170-220ms in camels ranging in age from <6 months to 16-18 years of age (Pourjafar *et al,* 2011b) and 160ms (range 60-200ms) (Al Khamis *et al,* 2023).

The QT interval represents the time it takes for the ventricles of the heart to depolarise and then repolarise, essentially the duration of ventricular systole (contraction). The QT interval was 382±5ms (n=8). Previous studies in camel have reported 380ms (range 340–440ms) (Al Khamis *et al,* 2023) and for comparison in cattle 220–480ms (Devadevi *et al,* 2022).

The T wave represents ventricular repolarisation, the process where the heart muscle cells in the ventricles return to their resting state after contracting. The T wave duration was 118±7ms (n=8). Previous studies in camel have reported 70–100ms in camels ranging in age from <6 months to 16–18 years of age (Pourjafar *et al,* 2011b) and for comparison in cattle 50–160ms (Devadevi *et al,* 2022).

The RR interval represents the time between two consecutive R waves, specifically the time between the peaks of the QRS complexes. The RR interval duration was 987±27ms (n=8). Previous studies in camel have reported 690–1100ms in camels ranging in age from <6 months to 16–18 years of age (Pourjafar *et al,* 2011b) and 1100ms (range 680–1310ms) (Al Khamis *et al,* 2023).

T/QRS ratio refers to the ratio of the amplitude of the T wave to the amplitude of the QRS complex. It's a parameter used in ECG analysis to differentiate between various cardiac conditions, particularly in the context of acute myocardial infarction and ventricular aneurysms. The T/QRS ratio was 26±2% (n=8).

The HR was 61±2 bpm (n=8). Previous studies have reported 48–89 bpm in camels ranging in age from <6 months to 16-18 years of age (Pourjafar *et al,* 2011b) and 60 bpm (range 48–96 bpm) (Al Khamis *et al,* 2023).

The HRV is the variation in time intervals between normal heartbeats as indicated by the R wave. These fluctuations, measured in milliseconds, are controlled by the autonomic nervous system and reflect rate changes due to cardiac demand. Since the study is based on 30 sequential beats, the short-term SDNN was used to estimate the HRV as 49±7ms (n=8).

It was interesting to observe the orientation of the various ECG waves (Fig 3). The P wave was consistently positive (Figs 4A–C), while the QRS complex had a negative deflection (Fig 4A) or biphasic (Figs 4B and 4C). The T wave was either positive (Figs 4A and 4B) or negative (Fig 4C). Electrode placement was consistent across all recordings. It is suggested that the recumbent position of the camel, along with shifting intrathoracic pressure, may have caused slight displacements of the heart, leading to variations in ECG wave orientation.

For interest, two examples of arrhythmia are shown in Fig 4: bradyarrhythmia (Fig 4A) and a tachyarrhythmia (Fig 4B). The causes of

Table 1. Summative Electrocardiogram Analysis.

Parameter	N	Maximum	Minimum	Mean	SEM	Median	IQR
Weight (kg)	8	550.0	405.0	495.6	18.5	501.0	59.9
Age (yr)	8	10.0	7.0	9.5	0.4	10.0	0.3
P Wave Duration (ms)	8	115	68	90	6	86	16
PR Segment (ms)	8	172	98	130	9	121	40
PR Interval (ms)	8	288	178	221	14	207	55
QRS Interval (ms)	8	95	70	79	3	77	13
ST Segment (ms)	8	216	163	185	6	178	23
QT Interval (ms)	8	410	360	382	5	380	16
T Wave Duration (ms)	8	142	80	118	7	119	24
RR Interval (ms)	8	1112	878	987	27	979	82
T/QRS Amplitude Ratio (%)	8	33%	17%	26%	2%	26%	4%
HR (BPM)	8	69	54	61	2	61	5
HRV (ms)	8	92	20	49	7	46	15

these arrhythmias remain unclear; however, the tachyarrhythmia may be stress-related.

This study provides comprehensive electrocardiographic measurements in clinically healthy adult female dromedary camels using modern recording and analysis techniques. The results confirm characteristic species-specific ECG features, including prolonged atrioventricular conduction and relatively low-amplitude QRS complexes and establish updated reference ranges for key parameters. The combination of sternal recumbency positioning and adhesive electrodes proved effective in reducing artifacts and improving signal quality. These findings enhance the understanding of camel cardiac electrophysiology and offer valuable benchmarks for clinical assessment and diagnosis of arrhythmias and conduction disorders in this species. Further research involving diverse age groups, breeds and physiological conditions will help to refine and expand normative ECG data to support veterinary care and management of camels across different settings.

Acknowledgements

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HAEMATOLOGICAL VARIATIONS BEFORE AND AFTER BLOOD TRANSFUSION IN ARABIAN RACING CAMELS (*Camelus dromedarius*)

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ABSTRACT

In this experiment, a haematological comparison has been done before and after whole blood transfusion in 5 racing camels (2 years old). The donated blood withdrawn from only one camel to fix the other variation factor from different blood from different donor camels. The variation was clear in some haematological parameters like red blood cells (RBC), haemoglobin (Hb), haematocrit (HCT), platelets (PLT). The reticulocytes count showed significant steady changes and could rely on this value as an indicator of blood transfusion in Arabian racing camels according to the results of this experiment and many other individual cases outside this experiment. The leukocytes and differential leukocyte percentages did not showed significant changes after blood transfusion. The immunological reactions against antigens on red blood cells of donor camel determine the variation in haematological changes after blood transfusion. However, the immunological reaction becomes with limited values especially in the first time of blood transfusion.

Key words: Blood transfusion, camel haematology, clinical pathology, racing camel

Fresh whole blood transfusion in livestock animals is indicated for the treatment of diseases associated with blood loss (Hunt and Wood, 1999; Divers, 2005). Whole blood transfusion becomes well known practice of camel racing in all Gulf countries last decade.

The benefits of blood transfusions are typically considered short term (Schnappauf *et al,* 1965; Kallfelz and Whitlock, 1973; Kallfelz *et al,* 1978). For example, bovine RBCs labeled with 59Fe decreased to 25% of their original concentration only 4 days after transfusion. After a second transfusion, 75% of the RBCs administered were absent within 30 minutes of administration (Kallfelz and Whitlock, 1973).

Blood transfusion is not always safe and reactions can occur due to improper compatibility, poor storage or faulty administration. In general immediate reactions occur within 2-4 hours post transfusions and delayed reactions may take days to weeks or even months (Sharma *et al,* 2000).

First blood transfusion has no untoward effect in cattle. But second or third transfusions may cause anaphylactic shock in animals (Chakrabarti, 2016). Delayed reactions can occur days to weeks after the administration of blood products. These reactions typically involve haemolysis caused by the formation of antibodies to RBC antigens and occur 3–5 days after transfusion (Hart, 2011). A retrospective study in foals with neonatal isoerythrolysis found that foals treated with more than 4 L of blood products were 19.5 times more likely to develop liver failure than those treated with a lower volume (Polkes *et al,* 2008).

Evaluation of the blood parameters are necessary before blood transfusion, packed cell volume (PCV) (≤ 15-20%) (Hunt *et al,* 1990; Hunt and Wood, 1999; Divers, 2005; Balcomb and Foster 2014) and haemoglobin concentration (Hb)(≤ 7g/dl) (Hebert *et al,* 2011) considered as a strong indication for blood transfusion. Whole blood transfusion is required in horses if packed cell volume (PCV) is less than 12%, haemoglobin concentration less than 8 g/dl, traumatic injury, haemophilia or a heavy infestation of Strongylus (Shatanu *et al,* 2019).

Present study was therefore done to evaluate the haematological parameters of Arabian racing camel before and after the blood transfusion.

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Materials and Methods

This Before-After study (Stewart-Oaten and Bence, 2001) was done in barns close to racing track in Dubai Emirate in UAE in 2020.

1. Donor camel:

Apparent healthy non-racing adult camel (average: 500 kg bw) was used as a donor camel to collect 4.5L blood without complications or health problem after blood collection.

2. Recipient Camels:

Five Arabian racing camels (*Camelus dromedarius*) around 2 years old kept in one camel barn, 2 km away from Camula Veterinary Laboratory in Marmoum, Dubai, UAE. Five ml of blood collected from Jugular vein in EDTA test tube from each camel, soon before blood transfusion. The total 5 test tubes were preserved immediately in ice box and were sent directly to the lab.

After two days from blood transfusion, 5 ml of blood collected from Jugular vein on EDTA test tube and it was sent directly to the lab for testing. The camels did not receive any medications or feed supplements during the two days.

3. Blood collection:

The process of collection and transfusion had been done within short time soon after blood collection.

3.1. Blood collection kit.

Plastic blood bags (450ml) containing CPDA (Mitra Industries Ltd. India).

3.2. Blood collection from the donor camels (Whole blood)

Blood was collected from the jugular vein of donor camel using 16G needle and blood collecting kit (450 ml). Ten bags were collected from donor camel. Donated blood preserved in isolated box

4. Blood transfusion process:

At the time of blood collection for the transfusion process a complete aseptic procedures were adopted. Blood was maintained at 37°C before transfusion. Blood was transfused intravenously through I/V filtering sets. Each camel received 2 bags (900 ml).

5. Blood Samples and Haematological tests

Total 10 blood samples, i.e. 5 samples immediately before blood transfusion and 5 samples after 2 days of blood transfusion were subjected to the haematological analysis by Advia®2120i haematology analyser (Semins Company, Germany). The blood samples were analysed for red blood cells (RBCs), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocyte deviation width (RDW), reticulocyte per cent (Retic. %), reticulocyte number (Retic. No.), platelets (PLT), mean platelet volume (MPV), platelets deviation width (PDW), plateletcrite (PTC), mean platelet concentration (MPC), white blood cells (WBCs), neutrophil % (Neut. %), lymphocyte % (Lymph. %), monocyte % (Mono. %), eosinophil % (Eos. %), basophil % (Baso. %).

6. Statistical Analysis

The obtained results of 5 camels were collected in excel sheet (Microsoft Office 10). The data was analysed using a statistical software programme (SPSS, version 16, USA). Paired sample repeated measure ANOVA was used to express means and standerd deviation and to evaluate for significant differences ($P < 0.05$) between before and after sample.

Results and Discussion

The changes in haematological parameter after blood transfusion were controlled by multiple factors including the volume of donated blood, which was represented by the equation:

Total Volume (TV) (Donated Blood) = Volume of Distribution of Blood (VDB) (Total circulating blood) x Body Weight (BW) kg x (Desired Packed Cell Volume (PCV) % - Recipient (PCV) % / Donor (PCV) % (Luethy *et al,* 2017).

Other factor like duration after blood transfusion plays an important role in the haematological changes. In this experiment, other factors were fixed (Donated blood from one donor camel, duration after transfusion (two days), avoiding using any medications or supplements).

Blood volume in camels is 93 ml per kg of body weight (Djegham and Belhadj, 1986), which is a higher value than that observed in most other domestic species. The stock blood volume must increase as we add 900 ml to the total volume.

There was no clinical finding after blood transfusion of all recipient racing camels within two days monitoring till collecting blood samples for testing the haematological parameters.

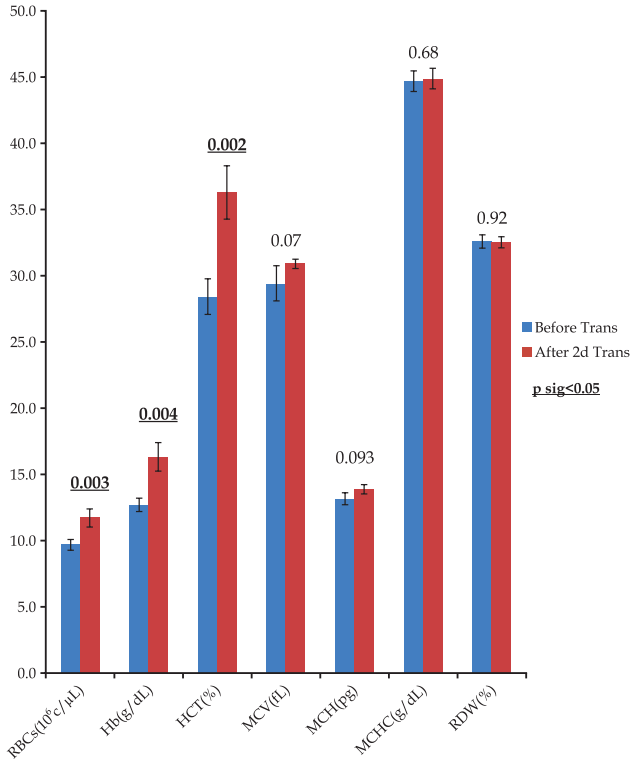

Fig 1. Haematological parameters (RBCs)—Comparison before blood transfusion and after 2 days in camels under the study.

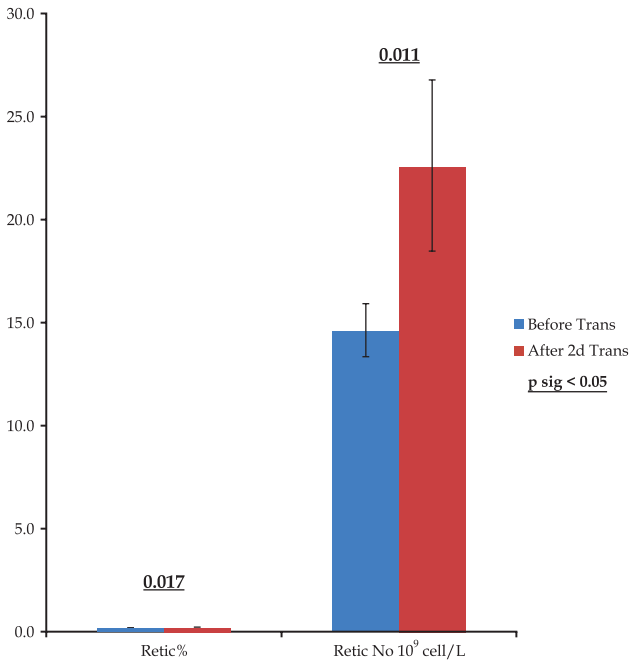

Fig 2. Haematological parameters (Reticulocytes)—Comparison before blood transfusion and after 2 days in camels under the study.

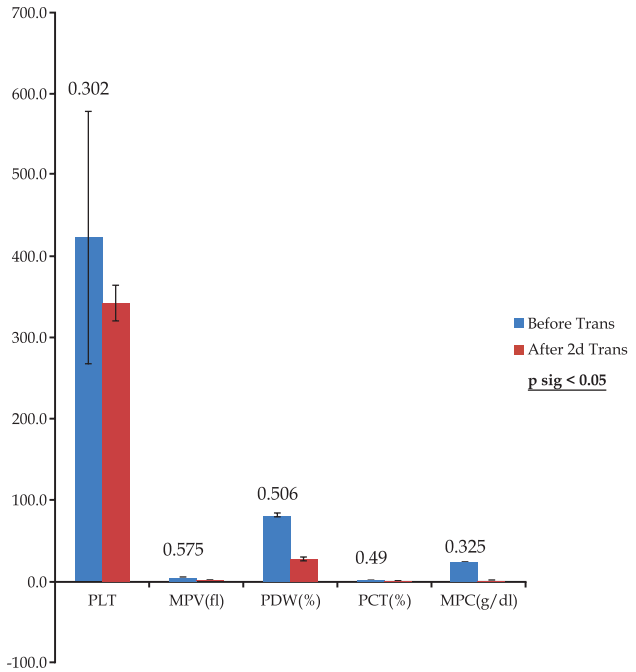

Fig 3. Haematological parameters (platelets)—Comparison before blood transfusion and after 2 days in camels under the study.

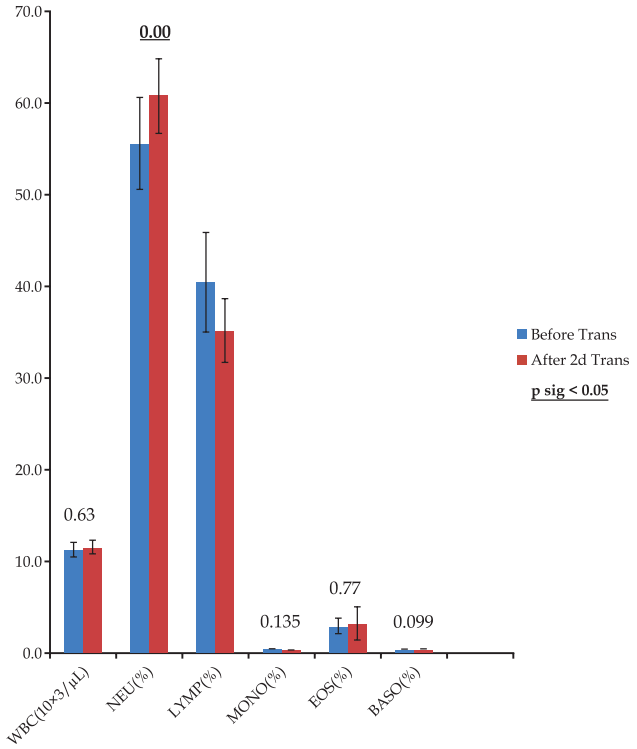

Fig 4. Haematological parameters (WBCs)—Comparison before blood transfusion and after 2 days in camels under the study.

Our findings was astonishing, it demonstrated that the significant changes were clear obviously in the RBCs, Hb, HCT, Plt and reticulocytes (Tables 1, 2 and 3).

It was impressive that the number of red blood cells RBCs showed clear and significant increase from 9.7 to 11.7 (10^6/µL) (Table 1), with average percent 20.6% (P-value 0.003) (Fig 1) The increase of RBC

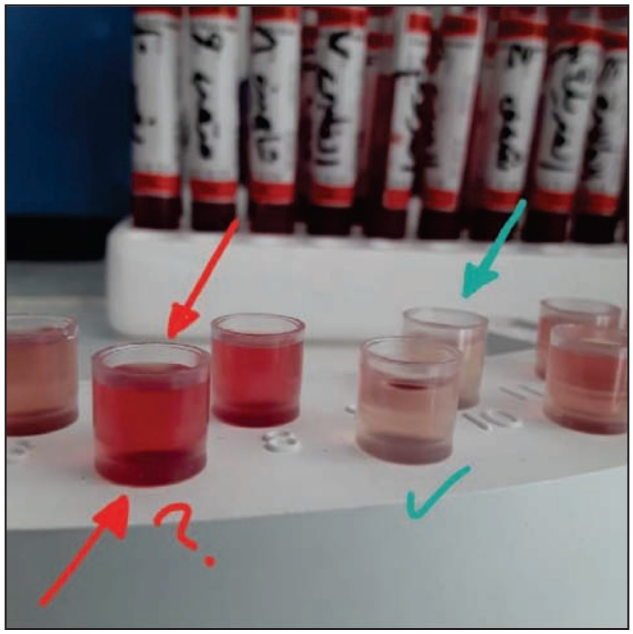

Fig 5. The image shows the difference in colour between the serum from different blood samples.

sometimes do not remain stable for long time because of short lifespan (Kallfelz and Whitlock, 1973) as the intravascular haemolysis lead to gradual decrease the total number of RBC, which was obviously clear in the colour of serum of blood samples coming from recipient Camels as shown (Fig 5), the image shows the difference in colour between the serum from different blood samples of recipient camels.

Table 1. Haematological parameters comparison before blood transfusion and after 2 days in camels under the study.

Parameter	Unit	Average Normal	Before Transfusion	After Transfusion	P-Value
RBCs	10^6c/µL	7 – 10	9.7 ± 0.43	11.7 ± 0.68	0.003
Hb	g/dL	10 – 15	12.7 ± 0.49	16.3 ± 1.1	0.004
HCT	%	25 – 33	28.4 ± 1.36	36.3 ± 2.04	0.002
MCV	fL	26 – 35	29.4 ± 1.32	30.9 ± 0.33	0.07
MCH	pg	12 – 17	13.1 ± 0.46	13.9 ± 0.34	0.093
MCHC	g/dL	40 – 50	44.7 ± 0.75	44.9 ± 0.82	0.68
RDW	%	30 – 33	32.6 ± 0.5	32.6 ± 0.42	0.92

Another cases in our study showed very high increase in all parameters related to RBCs. Additionally, the HCT percentage exceeded 45%. The trials failed to decrease the RBCs parameters and these remained within optimum range by using the fluid therapy and oral electrolytes to overcome the haemo-concentration. The response of immune system of recipient camel to the antigens on the surface of RBCs of donated blood determined the haematological values after blood transfusion.

Regarding HCT, our results showed significant increase from 28.4± 1.36% to 36.3± 2.04% (Table 1) which way around 28% more than the increased values in RBCs, because of increased values in the MCV, the changes in MCV probably related to the osmolarity changes of the blood related to dehydration (haemo-concentration) and rehydration (Yagil *et al*, 1974). Haemoglobin values recorded significant increase (exceeded 28%, P-value 0.004) (Fig 1), which may be related to intravascular haemolysis and this interfere with photometric method of Hb calculation by the analyser (Mezzou *et al*, 2006).

Concerning the immature RBCs, our data demonstrated significant changes in reticulocytes number and percentage and the changes were steady for long time after blood transfusion (Table 2); (Fig 2). Reticulocytes may provide useful information as they are present in the case of regenerative anaemia. Raisinghani *et al* (1981) observed no reticulocytes in healthy animals. However, in animals inoculated with *Trypanosoma evansi*, the reticulocyte rate may reach 6% after 88 days of infestation (Raisinghani *et al*, 1981) and even more than 11% in some cases (Jhatkar and Purohit, 1971). The trypanosomiasis is accompanied by hyperplasia of the bone marrow (Raisinghani *et al*, 1981), in consequence of the above-mentioned intravascular haemolysis, which explains the appearance of immature red blood cells in the peripheral circulation (Jhatkar and Purohit, 1971). The average total number of reticulocyte is (14.6±1.29) $\times 10^9$ cell/L in normal condition before blood transfusion, the total number increased in all camels in this experiment and many other individual cases exceed 20× 10^9 cell/L (Table 2). The increase in the total number of reticulocytes remains steady for long time and this could be used as indicator to the racing camels which received donated blood.

Table 2. Haematological parameters (Reticulocytes) – Comparison before blood transfusion and after 2 days in camels under the study.

Parameter	Unit	Average Normal	Before Transfusion	After Transfusion	P-Value
Retic	%	0 – 0.7*	0.15 ± 0.01	0.2 ± 0.03	0.017
Retic. No	10^9 cell/L	NA	14.6 ± 1.29	22.6 ± 4.17	0.011

* (Higgins and Kock, 1984)

Platelets count decreased significantly (thrombocytopenia) from (424 ± 156) to (342 ± 22)

cells x10^3/µL (Table 3) around 19% (P-value 0.302) (Fig 3) due to coagulation factors (Sharma *et al,* 2000). However, we can't judge this fact without monitoring for long time. The changes on PLT count usually vary according to the immunological reaction after blood transfusion. There is direct proportional between the intravascular haemolysis and platelets count at beginning due to adaptation of bone marrow to stop the RBCs loss, later on change to inverse proportion because of depletion of platelets related to intravascular coagulation and exhaustion of bone marrow production of new platelets (Satue *et al,* 2017). The other platelets parameters did not show significant changes (Table 3; Fig 3).

Table 3. Haematological parameters 9 (platelets)_Comparison before blood transfusion and after 2 days in camels under the study.

Parameter	Unit	Average Normal	Before Transfusion	After Transfusion	P-Value
PLT	10^3 cell/µL	150 – 500	424 ± 156	342 ± 22	0.302

On the other hand, our findings showed no significant differences in the leukocytes and its deferential cell percentages (Table 4; Fig 4). as we mentioned before that the reaction to a first transfusion is low (Divers, 2005; Andrews and Penedo, 2010; Balcomb and Foster, 2014). This exactly matches with the same results in this experiment, because this is the first time for these camels to receive donated blood. However, in some individual case outside this experiment showed sever changes in WBCs (leukocytosis) and deferential percentage accompanied with severe neutrophilia (Table 4; Fig 4).

Table 4. Haematological Parameters (WBCs)—Comparison before blood transfusion and after 2 days in camels under the study.

Parameter	Unit	Average Normal	Before Transfusion	After Transfusion	P-Value
WBC	10^3 cell/µL	8 – 15	11.2 ± 0.83	11.5 ± 0.69	0.63
Neut	%	40 – 60	55.6 ± 5.04	60.8 ± 4.09	0.00
Lymph	%	25 – 45	40.4 ± 5.42	35.2 ± 3.49	0.117
Mono	%	0 – 8	0.5 ± 0.08	0.3 ± 0.11	0.135
Eosino	%	0 – 6	3 ± 0.81	3.2 ± 1.83	0.77
Baso	%	0 – 1	0.4 ± 0.05	0.4 ± 0.09	0.099

*Optimum values of female Arabian racing camel (2 years old) (Elhag *et al,* 2016).

The haematological parameters showed significant changes in some parameters after blood transfusion of Arabian racing camels. RBCs, Hb, HCT showed significant increase (20%, 28.4% and 28%, respectively). While platelets count showed significant decrease around (19%). The leukocytes and its deferential cell percentages did not show any significant variation. Blood transfusion in Arabian racing camels required more scientific trials to determine the blood grouping and more understanding of the immunological reaction after blood transfusion. The blood transfusion must be with very limited veterinary practice in severe cases of anaemia and must be under direct supervision of veterinarian.

Acknowledgement

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APPLICATION OF BACTRIAN CAMEL DERIVED NANOBODIES IN THE DETECTION OF FOODBORNE PATHOGENS

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ABSTRACT

Foodborne pathogens pose a significant threat to public health and the limitations of traditional detection methods have underscored the urgent need for rapid and sensitive novel technologies. Nanobodies (Nbs), owing to their unique structural features, exhibit substantial potential for the detection of foodborne pathogens. This review systematically examines the structural characteristics, biological properties, advantages as detection tools and preparation methods of Bactrian camel-derived Nbs. It elaborates on their applications in detecting various foodborne pathogens, including Salmonella, *Cronobacter sakazakii* (*C. sakazakii*) and *Vibrio parahaemolyticus* (*V. parahaemolyticus*). Nb-based technologies such as enzyme-linked immunosorbent assay (ELISA), immunochromatographic test strips and colourimetric sensors have demonstrated high sensitivity, specificity and rapid detection capabilities. Meanwhile, this paper analyses the current challenges, such as insufficient antibody affinity and non-specific binding in complex matrices and looks forward to future directions, including modification of antibodies and development of integrated detection platforms in combination with emerging technologies. This provides a reference for in-depth research and application of Nbs in foodborne pathogen detection.

Key words: Bactrian camel, food safety, foodborne pathogens, immunoassays, nanobodies

Illnesses caused by foodborne pathogens pose a serious threat to global public health, with a large number of people falling ill or even dying each year from consuming contaminated food. Foodborne pathogens are reported to cause approximately 600 million cases of illness and 420,000 deaths worldwide annually (Maguire Van Seventer and Hamer, 2017). Traditional methods for detecting foodborne pathogens, such as microbial culture, remain the gold standard but are time-consuming, low-throughput and inefficient (Hendrickson *et al,* 2019). Molecular biology methods, although accurate, specific and sensitive, require specially designed probes, cumbersome sample preparation and are prone to false positives. They are also limited by the need for expensive equipment and skilled operators (Mandal *et al,* 2011; Zhang *et al,* 2020). With the rapid pace of the food supply chain, there is an urgent need for rapid, sensitive and accurate detection technologies to enable early monitoring and control of foodborne pathogens.

Immunoassays based on the specific antigen-antibody reaction, characterised by simple operation, high accuracy and rapid detection, have been developing rapidly and playing an increasingly important role in the detection of foodborne pathogens. As an emerging immune biomolecular tool, Nbs have emerged in the field of foodborne pathogen detection in recent years. Their unique structure confers many excellent properties, providing a new approach to addressing the bottlenecks of traditional detection techniques. As an important source of Nbs, Bactrian camel, due to its special physiological adaptations and immune properties, produces Nbs with high diversity and unique functions. The application of Bactrian camel-derived Nbs in foodborne pathogen detection is of great significance for enhancing food safety and protecting public health.

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2. Overview of nanobodies

2.1. Structural features of nanobodies

Antibodies in camelids differ from those of other mammals in that they contain two naturally occurring structural antibodies, a conventional tetrameric antibody and a dimeric antibody composed solely of heavy chains, referred to as the heavy chain antibody (HCAb) (Hamers-Casterman *et al,* 1993). Although the HCAb lacks the light chain and the first constant region of the heavy chain (CH1), it remains functionally intact as an antibody. Upon cloning the variable domain of HCAb in camel, a single domain antibody called VHH or Nb was obtained. It has a diameter of 2.5 nm, a length of 4 nm and a molecular weight of only 15 kDa—merely one-tenth that of a conventional antibody. This makes it the smallest genetically engineered antibody with full antigen-binding functionality (Muyldermans, 2013; Hassanzadeh-Ghassabeh *et al,* 2013; Jin *et al,* 2023).

Nb consists of four framework regions (FRs) and three complementarity determining regions (CDRs). The variable region of the human heavy chain (VH) shares over 80% homology with VHH, yet exhibits significant differences (Arbabi Ghahroudi *et al,* 1997). VH contains six CDRs in total, whereas VHH possesses only three, with CDR1 and CDR3 being longer in VHH. The extended CDR1 and CDR3 in VHH compensate, to some extent, for the absence of light chains in antigen-binding capability. The convex loop structure formed by CDR3 facilitates antigen binding, enabling VHH to recognise cryptic antigenic epitopes more effectively (De Genst *et al,* 2006). The FR2 of VH harbours four highly conserved hydrophobic amino acids (V42, G49, L50, W52), which are substituted by four hydrophilic amino acids (F42, E49, R50, G52) in VHH. This substitution significantly enhances VHH hydrophilicity and improves its water solubility (Vu *et al,* 1997; Harmsen *et al,* 2000). In addition, both FR1 and FR3 contain a cysteine that forms a disulfide bond, conferring greater stability to Nb and enabling it to withstand extreme temperatures, pH ranges and chemical reagent treatments (Muyldermans *et al,* 1994). Furthermore, Nbs may form additional disulphide bonds at CDR1-CDR3, FR2-CDR3 and CDR3-CDR3, further enhancing their structural stability (Melarkode Vattekatte *et al,* 2020).

2.2. Biological properties of nanobodies

2.2.1. High stability and water solubility

Owing to the absence of light chains and CH1, Nbs exhibit more compact intramolecular interactions and multiple internal disulfide bonds, rendering their molecular structure relatively stable. This stability enables them to maintain activity in extremely harsh environments and at high temperatures (Li *et al,* 2018). Compared with conventional antibodies, Nbs show greater stability in complex detection environments, a feature that ensures the reliability of Nb-based detection reagents during storage and transport. Hydrophilic amino acids in the VHH increase the water solubility of the Nb when replacing hydrophobic amino acids exposed on the surface of the VH (De Genst *et al,* 2013). This high water solubility allows the Nbs to remain uniformly dispersed in complex solvents, avoiding the problem of activity loss due to hydrophobic aggregation in conventional antibodies.

2.2.2. High affinity and specificity

Nbs with long CDR3 and highly diverse sequences can form unique antigen-binding sites, enabling them to recognise antigenic epitopes that are inaccessible to conventional antibodies. This significantly enhances the accuracy and sensitivity of detection (De Genst *et al,* 2006). The loop structure of CDR3 improves antigen-antibody binding capacity, thereby enhancing the specificity and affinity of Nbs for antigens.

2.2.3. Low immunogenicity and simple humanisation

Nb has a simple structure and lacks a fragment crystallisable (Fc), which avoids the complement reaction caused by Fc contained in conventional antibodies. Moreover, they have high homology with human VH and can be easily cleared *in vivo* and can be humanised by simple modification of Nb (Liu *et al,* 2025). In detection systems, Nbs are less prone to trigger immune rejection or non-specific binding, effectively reducing background signals. This feature makes them particularly suitable for detecting foodborne pathogens in complex food matrices, as it minimises interference from food components and enhances detection reliability.

2.2.4. Small molecular weight and tissue penetration

With a molecular weight of only about 15 kDa, Nbs are able to rapidly penetrate the tissue interstitium and extracellular matrix (Arbabi-Ghahroudi, 2017). When detecting pathogenic bacteria that are wrapped in food matrix or hidden in the deeper layers of tissues, Nbs can rapidly reach the target location and bind to it, breaking through the spatial site-blocking limitation of conventional antibodies due to the larger molecules, which facilitates rapid detection.

2.2.5. Easily modified by genetic engineering

Nbs are encoded by a single gene, enabling genetic engineering to readily introduce tags, enzyme molecules, or other functional domains for constructing diverse assay systems. For instance, conjugating Nbs with nanoparticles (Zhang *et al,* 2022a) or magnetic beads (Bai *et al,* 2023) facilitates the development of detection technologies such as immunochromatography and immunomagnetic separation (IMS). Fusing Nbs with reporter enzymes (Gu *et al,* 2022) enables signal amplification in ELISA or biosensors. Additionally, directed evolution and structural modification (Wang *et al,* 2023a; Liao *et al,* 2024) can further optimise Nb properties, such as enhancing stability, affinity, or altering specificity.

2.3. Preparation of Nanobodies

2.3.1. Nanobody libraries

Nb libraries can be classified into naïve libraries, immune libraries and semi-synthetic/synthetic libraries. A naïve library is constructed by directly extracting mRNA from peripheral blood lymphocytes of unimmunised Bactrian camels and cloning variable domain genes without immune antigens. This library contains the full diversity of Nb genes naturally present *in vivo*. The method saves time and cost, enabling screening for toxic, weakly immunogenic, or hardly preparable antigens (Tu *et al,* 2016). However, screening is relatively challenging, requiring extensive efforts and ensuring the library has sufficient capacity and diversity to isolate Nbs with high specificity and affinity against foodborne pathogens.

In contrast, immune libraries are more targeted: immunising Bactrian camels with specific foodborne pathogens stimulates *in vivo* B cell proliferation to produce specific Nbs, thus avoiding *in vitro* affinity maturation (Lim *et al,* 2014). Immune libraries do not require large capacity to screen high-affinity and stable Nbs. However, each antigen necessitates re-immunisation and library construction, leading to a lengthy and costly cycle.

Library capacity and diversity are key factors affecting antibody screening. By modifying base sequences and constructing synthetic/semi-synthetic libraries completely or partially *in vitro*, the FRs and CDRs of Nbs can be designed and mutated as needed. Introducing diversified sequences enhances library diversity and capacity, also enabling customisation of Nb properties to meet special detection requirements—such as improving stability or altering specificity (Liu *et al*, 2018; Valdés-Tresanco *et al*, 2022; Liu *et al*, 2024).

2.3.2. Screening techniques for nanobodies

The screening of Nbs primarily involves phage display, ribosome display and yeast surface display techniques. Among these, phage display technology is the most widely used. Its principle lies in introducing exogenous genes into a phage vector, which are expressed as fusions with phage coat proteins and displayed on the phage surface (Sidhu, 2001). The collection of all phages displaying exogenous genes is termed a phage display library. High-affinity Nbs can be screened via biopanning, leveraging the specific binding of antigens to Nbs, making it commonly used for the initial screening of specific Nbs against foodborne pathogens.

Ribosome display technology is a cell-free screening method that does not depend on the transformation and expansion process of cells. It is based on the principle that transcription and translation are performed *in vitro* so that the VHH gene, mRNA and ribosomes form a stable complex, enabling the display of Nbs on the ribosome surface (Bencurova *et al,* 2015). This technique allows for rapid *in vitro* evolution and screening of Nbs and is particularly suitable for studies where affinity is optimised.

Yeast surface display uses yeast as a display vector, fusing specific VHH genes with yeast-secreted proteins to display Nbs on yeast cell surfaces (McMahon *et al,* 2018). Compared to phage display libraries, yeast display libraries allow for more complex post-translational modifications of expressed Nbs, aiding in forming the correct spatial conformation. This makes them suitable for Nb screening with high stability and activity requirements (Ryckaert *et al,* 2010).

With the development of sequencing technology, single-cell sequencing technology has been gradually applied to Nb screening. This technology is capable of directly sequencing individual B cells to obtain their antibody gene sequences. In the screening of Nbs against foodborne pathogens, individual B cells can be isolated from immunised Bactrian camel lymphocytes and their VHH gene sequences can be analysed by single-cell sequencing technology, so that potentially specific Nb genes can be screened out by bioinformatics analysis (Yi *et al,* 2024). Single-cell sequencing technology can avoid the bias during library construction and directly obtain the natural Nb sequences, which provides a new way to screen highly specific Nbs.

2.3.3. Preparation process of nanobodies

The conventional procedure for preparing Nbs involves isolating peripheral blood lymphocytes from Bactrian camels immunised or unimmunised against specific foodborne pathogens, extracting total RNA, reverse-transcribing it into cDNA, amplifying the VHH gene fragment with specific primers, cloning it into phage vectors, transforming it into *Escherichia coli* (*E. coli*) cells and constructing a phage-displayed Nb (phage-Nb) library with the aid of helper phages. Phage-Nbs that specifically bind to the target antigen are screened through 3–4 rounds of adsorption, washing, elution and amplification using solid-phase biopanning. Finally, the Nbs are obtained by prokaryotic induction expression, affinity chromatography purification and activity determination, meeting the requirements for foodborne pathogen detection (Gavira-O'Neill *et al,* 2020; Zhang *et al,* 2021).

3. Application of nanobodies in the detection of foodborne pathogens

3.1. Enzyme-linked immunosorbent assay based on nanobodies

ELISA is one of the most widely used immunoassays and foodborne pathogens are typically detected by sandwich ELISA. Pathogens are first captured by a capture antibody and then reacted by horseradish peroxidase (HRP)-conjugated antibody as a detecting antibody with 3,3′,5,5′-tetramethylbiphenyl (TMB) to produce a measurable colour change (Chunglok *et al,* 2011). In the field of foodborne pathogen detection, ELISA based on Bactrian camel Nbs has emerged as a premier research direction due to its technical maturity and application potential (Table 1).

He *et al* (2020) established a sandwich ELISA for the detection of *Salmonella enteritidis* (*S. enteritidis*) by using a polyclonal antibody (pAb) as the capture antibody and Nb13 as the detection antibody, with a limit of detection (LOD) was 1.4×10^5 CFU/mL. The method was suitable for milk samples and 6 CFU/mL of *S. enteritidis* could be detected after 10 h of enrichment. However, in the development of sandwich ELISA, Nb is mostly used as detection antibody and pAb or monoclonal antibody (mAb) as capture antibody. This is due to the difficulty of pairing dual Nb in a limited number of Nb. In addition, the small size of Nb may hinder the fixation of binding sites on polystyrene microtitre plates. Some studies have reported that the affinity constants of Nb for antigens are typically 10^2–10^3 times lower than those of intact antibodies from immunised animals.

Notably, Gu *et al* (2022) established a sandwich ELISA for the detection of *S. enteritidis* in milk by genetic manipulation using SE-Nb9 as the capture antibody and SE-Nb1-vHRP as the probe. The method reduces the use of commercially available secondary antibodies, shortens the time of the assay and reduces the cost and can detect *S. enteritidis* as low as 5×10^4 CFU/mL. In order to efficiently avoid the interference of food matrices, to shorten the enrichment period and to improve the detection sensitivity. Phage display technology is a powerful and commonly used tool to amplify the signal and improve the sensitivity of the assay by using phage-Nb as a detection antibody to compensate for the lack of Nbs in affinity. Zhang *et al* (2022b) established phage-mediated dual Nb sandwich chemiluminescent enzyme immunoassay. The chemiluminescence reaction was used to replace the traditional colour development reaction and a highly sensitive detection of *Salmonella typhimurium* (*S. typhimurium*) was achieved with a LOD of 3.63×10^3 CFU/mL, which was 100-fold higher than that of the traditional Nb-ELISA.

Multivalent modification of Nbs has been investigated to further improve sensitivity, aiming to balance stability with enhanced affinity by increasing Nb size. Liao *et al* (2024) established a sandwich ELISA based on bivalent Nb (BNb-ELISA), with a LOD of 2.364×10^3 CFU/mL against *S. enteritidis*, which was a 7.5-fold improvement over the monovalent Nb ELISA. In addition, Bai *et al* (2023) developed a Nb-based sandwich ELISA combined with an IMS (IMS-ELISA) for the rapid enrichment and detection of *S. enteritidis* in food products. The IMS-ELISA achieved an LOD of 3.2×10^3 CFU/mL and reduced pre-enrichment time by 2 h in real sample analysis, effectively avoiding matrix interference and demonstrating potential for food pathogen monitoring. In summary, Bactrian camel Nb-based ELISA has continuously enhanced sensitivity and specificity in foodborne pathogen detection, progressing from laboratory research to rapid, precise food safety monitoring through antibody optimisation, signal amplification and pretreatment innovation.

3.2. Other immunoassays based on nanobodies

Driven by detection technology advancements and market demands, the field of high-throughput, on-site rapid detection of foodborne pathogens has garnered significant attention (Table 2).

Immunochromatographic technology integrates thin-layer chromatography with immune recognition, offering advantages of speed, simplicity, low cost and requiring no specialised personnel or sophisticated equipment (Gondhalekar *et al*, 2020). However, traditional immunochromatographic test strips are facing significant challenges, such as insufficient sensitivity and the inability to conduct real-time quantitative detection.

Zhang *et al* (2022a) developed KMO@Au and Nb9-assisted colourimetric photothermal dual-mode immunochromatographic test strips for rapid, sensitive and quantitative detection of *S. typhimurium* in food. The small size of Nb9 enabled efficient conjugation with flower-like KMO@Au photothermal agents, synergistically enhancing detection sensitivity and specificity. This biosensor achieved a LOD of 10^4 CFU/mL in colourimetric mode and 10^3 CFU/mL in photothermal mode, with successful application to real sample analysis demonstrating high precision. Preparing nanoparticle-Nb conjugates with high stability and affinity remains a key challenge in lateral flow immunoassay (LFIA) development. Wang *et al* (2025) enhanced the bioactivity of gold-Nb nanoprobes by directionally conjugating biotinylated Nb9 with streptavidin-coated gold nanoparticles (AuNPs). The resulting Au/SA@Bio-Nb probe exhibited exceptional stability and affinity, enabling

Table 1. Enzyme-linked immunosorbent assay based on nanobodies for the detection of foodborne pathogens

Immunoassay	Foodborne pathogen	Capture antibody+Detection antibody	LOD (CFU/mL)	Reference
Traditional sandwich ELISA	*Listeria monocytogenes* (*L. monocytogenes*)	mAb+Nb	1×10^4	Tu *et al* (2016)
Traditional sandwich ELISA	*S. enteritidis*	pAb+Nb	1.4×10^5	He *et al* (2020)
Traditional sandwich ELISA	*E. coli* O157:H7	pAb+Nb	8.7×10^3	He *et al* (2025)
Sandwich ELISA without additional secondary antibodies	*S. enteritidis*	Nb+HRP-Nb	5×10^4	Gu *et al* (2022)
Divalent modified sandwich ELISA	*Salmonella*	Divalent Nb+Phage-Nb	2.364×10^3–1.501×10^4	Liao *et al* (2024)
Streptavidin-bridged sandwich ELISA	*Salmonella*	Biotinylated Nb+ Phage-Nb	4.23×10^3–9.15×10^3	Ren *et al* (2022)
Bispecific modified sandwich ELISA	*S. enteritidis V. parahaemolyticus*	Bispecific Nb+Phage-Nb	3.33×10^3-6.35×10^4	Wang *et al* (2023a)
Chemiluminescent sandwich ELISA	*S. typhimurium*	Nb+Phage-Nb	3.63×10^3	Zhang *et al* (2022b)
Chemiluminescent sandwich ELISA	*C. sakazakii*	Nb+Phage-Nb	1.04×10^4	Zhang *et al* (2023)
Immunomagnetic sandwich ELISA	*S. enteritidis*	Nb+Phage-Nb	3.2×10^3	Bai *et al* (2023)

Table 2. Other immunoassays based on nanobodies for the detection of foodborne pathogens

Immunoassay	Foodborne pathogen	Capture antibody+Detection antibody/Detection antibody	LOD (CFU/mL)	Reference
Colourimetric and photothermal dual-mode immunochromatography biosensor	*S. typhimurium*	mAb+Nb	Colourimetric: 10^4 Photothermal: 10^3	Zhang *et al* (2022a)
Colourimetric immunosensor	*V. parahaemolyticus*	Phage-Nb	Visual: 10^4 Quantitative: 10^3	Wang *et al* (2023b)
Chromogenic immunosensor	*C. sakazakii*	Nb	Visual: 10^3 Quantitative: 13^6	Chen *et al* (2024)
Streptavidin-biotin immobilised lateral flow immunoassay	*S. typhimurium*	pAb+Biotinylated Nb	Visual: 10^3	Wang *et al* (2025)

an improved LFIA for rapid, sensitive *S. typhimurium* detection. The Au/SA@Bio-Nb-LFIA achieved a visual LOD of 10^3 CFU/mL and a linear detection range of 10^3–10^7 CFU/mL.

Notably, the above methods rely on paired antibodies, limiting their application in foodborne pathogen detection. Thus, establishing immunoassays based on single recognition elements is crucial for real-time pathogen monitoring. Wang *et al* (2023b) developed a simple, sensitive colourimetric immunosensor for *V. parahaemolyticus* by leveraging thiolation of phage-Nb (phage-Nb-SH) on pVIII shell proteins to induce AuNP aggregation. Specific interactions between nanomolecules and bacteria prevented aggregation, altering surface plasmon resonance and triggering a visible colour change. The assay completed within 100 min, with a visual LOD of 10^4 CFU/mL and quantitative LOD of 10^3 CFU/mL, showing no cross-reactivity with other bacteria. Innovations from traditional immunochromatography to novel colourimetric methods, combined with Nbs and advanced materials, are driving foodborne pathogen detection toward high-throughput, high-sensitivity and on-site capabilities. These advancements break free from antibody-dependency limitations, providing diversified solutions for enhancing rapid food safety detection systems.

4. Conclusion and Outlook

Nbs, with their unique structural and performance advantages, have made significant progress in foodborne pathogen detection, offering a series of efficient, rapid and sensitive methods for food safety analysis. In practical applications, Nb-based detection technologies effectively address the limitations of traditional methods, playing a critical role in ensuring food safety by enabling rapid screening and accurate quantification of foodborne pathogens throughout food processing and distribution.

However, the application of Nbs in the detection of foodborne pathogens still faces some challenges. On the one hand, the large-scale production process of Nbs needs to be further optimised to reduce the cost and increase the yield to meet the increasing demand for detection. Currently, although a variety of expression systems have been used for Nb production, there is still room for improvement in yield and cost control. On the other hand, traditional phage display library screening requires multiple rounds of biopanning, with a long cycle and high antigen purity requirements. Complex pathogenic bacterial antigens (e.g. surface polysaccharides, protein complexes) may lead to insufficient affinity or specificity crossover of the screened Nbs. In addition, some Bactrian camel Nb-based detection techniques have long detection times, require supporting instrumentation and have limited on-site rapid detection capabilities; while certain methods are portable but have low sensitivity, making it difficult to meet trace contamination detection needs. Finally, although Nbs have good specificity, a certain degree of non-specific binding may still exist in complex food matrices, leading to elevated background signals or false-positive results affecting detection accuracy. Although Nbs have significant advantages in the detection of foodborne pathogens, they still face many challenges and need to make further breakthroughs in technological innovation and system optimisation.

With the continuous development of biotechnology, Nbs in foodborne pathogens detection will usher in a broader development prospect. In terms of technological innovation, an integrated and intelligent detection platform can be developed by combining emerging nanotechnology and microfluidic technology. For example, combining Nbs with nanosensors and microfluidic chips to build a portable, high-throughput rapid detection system for foodborne pathogens, which can achieve simultaneous, rapid and accurate detection of multiple pathogenic bacteria. Meanwhile, we will further expand the application of Nbs in the detection of new foodborne pathogens and in the monitoring of the whole food supply chain, so as to provide more comprehensive and reliable technical support for ensuring food safety.

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FIRST PHENOTYPIC CHARACTERISATION OF LOCAL DROMEDARY CAMEL ECOTYPE IN EL OUED REGION, SOUTHEAST ALGERIA

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ABSTRACT

Understanding morphological variation among regional camel ecotypes is essential for documenting genetic resources and supporting effective conservation and breeding efforts. This study was aimed to phenotypically characterise dromedary camels in El Oued Province, southeastern Algeria, to understand the diversity and distribution of qualitative traits within the local camel ecotype. A total of 63 camels were randomly selected for phenotypic assessment. The qualitative traits evaluated included the ecotype of the animal; the colour of the coat, eyes, and muzzle; pigmentation of the eyelid, ear, and foot; the orientation of the ear and hump; the shape and position of the hump; and the facial and back-line profiles. Statistical analyses were conducted to determine the frequency distribution of each trait. The Arbia breed predominated, representing 65.08% of the ecotype, followed by Tergui and Zegria breeds. Nine distinct coat colours were identified, with Ahdjel being the most prevalent (23.81%). Brown eyes were the most common (85.71%), while blue eyes were rare (1.59%). Muzzle pigmentation was nearly evenly split between pigmented (52.38%) and non-pigmented (47.62%). Hump size varied, with small humps being the most frequent (65.08%), and all camels exhibited upright hump orientations. Additionally, all individuals had straight facial profiles and straight back-line profiles, with centrally positioned humps. This study provides valuable insights into the phenotypic diversity of dromedaries in the El Oued region, highlighting the significant roles of environmental adaptation and herder preferences in shaping the characteristics of local camel ecotypes, thereby informing future conservation and breeding programs.

Key words: Adaptation, camel, colour, ecotype, pigmentation

In Algeria, camels play a pivotal socio-economic role, particularly within El Oued Province in the southeast, where they adapt to the challenging desert environment characterised by extensive dunes and sparse vegetation (Harek *et al*, 2022). According to the latest FAOSTAT data, Algeria's camel population was estimated at 459,616 heads in 2022. The distribution of dromedary camels in Algeria is primarily concentrated in three regions: the central Sahara, which accounts for 56% of the national livestock, followed by the Northern Sahara (37%) and the Steppe (7%). The main camel breeds first identified in Algeria include Chaambi, Ouled Sidi Cheikh, Ait Khebbach, Steppe camel, Saharaoui (Arbia), Targui, Ajjer, Reguibi, and Ftouh (Aissa, 1989).

Phenotypic characterisation involves the assessment of observable traits such as coat colour, eye colour, hump shape, and other morphological features, providing insights into genetic diversity, adaptability, and breed differentiation (Ibtissam *et al*, 2023; Meghelli *et al*, 2020). The extent of phenotypic variation is valuable for selecting and utilising different camel ecotypes based on their specific characteristics and body conformation in breeding programs (Yosef *et al*, 2014). Despite their importance, limited research has focused on the phenotypic diversity of local camel ecotypes in Algeria, which is crucial for sustainable breeding programs and conservation efforts. There remains a notable gap in the literature regarding the phenotypic characterisation of Algerian dromedary camels, particularly in the El Oued region.

This study aims to provide the first comprehensive phenotypic characterisation of the

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local dromedary camel ecotype in El Oued Province. By examining qualitative traits, this research seeks to elucidate the diversity and distribution of phenotypic characteristics among camels in this region. The findings will contribute to a better understanding of how environmental adaptation and herder preferences shape the phenotypic landscape of Algerian camels, thereby informing future conservation and breeding programmes.

Materials and Methods

Study Area

The research took place in the province of El Oued, situated in southeastern Algeria at an altitude of 88 meters. It is located at 33°21′ N latitude and 6°51′ E longitude (Fig 1). The region's southern area is characterised by dunes, while the northern section features a sandy desert with sparse vegetation and a Salt Lake, known as a chott, to the west (Ramdani *et al*, 2022). The cold season lasts from November to April, with January temperatures falling to a minimum of 11.3°C. Conversely, the hot season runs from May to September, with August temperatures reaching a maximum of 33.91°C. The yearly average temperature in the area is approximately 22.47°C. The El Oued region's vegetation typifies Saharan flora, including xerophytic plants, perennials, woody species, and annuals with brief vegetative phases and well-developed root systems (Chergui *et al*, 2023).

Studied Animals

This study was conducted across 21 farms located in various regions of the Wilaya of El Oued, involving 19 distinct breeders. A total of 63 camels representing the local ecotype were examined, distributed among the five selected study areas: Mih Ouenssa, Oued Alenda, Douar Elma, El Oued, and Robbah. Although camels were initially sampled randomly, the final selection of specific animals was determined by the breeders. The primary inclusion criterion was age, with only dromedaries older than 5 years for females and 7 years for males being measured. This age threshold was established to ensure that all camels had reached full maturity, thereby providing more reliable and consistent phenotypic data for analysis.

Phenotypic Characterisation

Phenotypic characterisation was conducted through systematic visual assessments and photographic documentation. The qualitative traits evaluated included:

- Coat Colour: Variations in the overall colour of the camel's coat.
- Eye Colour: Differences in the colour of the eyes.
- Eyelid Pigmentation: Patterns and colours present on the eyelids.
- Ear Orientation: The angle and direction in which the ears are set.
- Hump Shape: The form and structure of the camel's hump.
- Hump Orientation: The positioning of the hump relative to the body.
- Hump Position: The vertical placement of the hump on the back.
- Facial Profile: The outline of the camel's face when viewed from the side.
- Muzzle Colour: The colouration of the muzzle area.
- Hair Length: The length of hair in specific regions of the body.
- Back Line Profile: The outline of the camel's back when viewed from the side.
- Ear Pigmentation: Colour patterns present on the ears.
- Foot Pigmentation: Colouration patterns on the camel's feet.

These traits were meticulously recorded to facilitate an in-depth analysis of phenotypic diversity, adaptability, and potential breed differentiation within the local camel ecotypes.

Statistical Analysis

The data collected from phenotypic assessments were processed to generate descriptive statistics, focusing on the frequency distribution of each phenotypic variable. Specifically, frequencies and percentages were calculated to quantify the prevalence of each trait category among the studied camels. All statistical analyses were performed using R software (R x64 3.1.0)

Result and Discussion

Understanding the phenotypic diversity and distribution of camel ecotypes is essential for optimising breeding strategies and enhancing livestock productivity in arid regions. This study focuses on El Oued region, a significant area for camel rearing, to analyse the prevalence of different camel breeds and their qualitative variations.

The data represents dromedary camel ecotypes distributed in the El Oued region (Table

1). Arbia ecotype is clearly dominant at 65.08%. This finding is particularly intriguing when compared to Aouachria's (2020) report, which indicated an even higher prevalence of the Arbia breed at 92.8%. The substantial representation of the Arbia ecotype in this recent study may be attributed to several factors. One significant aspect to consider is the role of high milk production attributed to the Arbia breed, which is often a key driver for farmers' preferences in livestock selection. The superior lactation performance of Arbia camels likely enhances their desirability among pastoralists and agricultural stakeholders in the region, leading to increased herd sizes and a higher concentration of this breed. In contrast, Tergui ecotype, which makes up 30.16%, and the Zegria ecotype at 4.76% demonstrate a much lower prevalence. This variation suggests potential shifts in breeding practices, regional environmental adaptations, and dairy production that may favour the Arbia breed's genetic characteristics in El Oued.

Table 1. Dromedary camel ecotypes distributed in the El Oued region.

Parameter	Modality	Percentage (%)
Ecotype	Arbia	65.08
	Tergui	30.16
	Zegria	4.76

Fig 2 articulate the diverse coat colour distribution among dromedaries in El Oued region, revealing a total of nine distinct coat colours, with dominance in Ahdjel (23.81%), Ahmer (19.05%), Asfer (14.29%), and Abyad (12.70%) identified as the most dominant (Table 2). One interesting finding is the seasonal change in camel coat colour, with dromedaries becoming lighter in warm months and darker in cold ones. This suggests they adapt to different climates for survival. Additionally, herders use the stable head colour to identify camels, since head colour does not change with the seasons. This highlights the importance of consistent traits for easier identification and breeding. The predominance of Ahdjel as the most frequent coat colour in this study contrasts sharply with Oulad Belkhir's (2018) findings from the northern Sahara, where Ahmer dominated at 60.28%. According to Bouregba and Lounis (1992), the majority of the ecotypes of the northern Sahara are red (brown Ouber), whereas Arif and Regab (1995) show that the dromedaries of the northern Sahara are of several natural colours such as Ahmar (red), Asfar (yellow), Abayd (white), and Azrek (blue). This discrepancy may stem from regional environmental factors, localised breeding practices, or differing management strategies that affect camel ecotype structures. The relative decrease in Ahmer in the El Oued region, despite maintaining a significant presence at 19.05%, suggests that herders here may be adapting their breeding selections based on factors beyond aesthetics, including practical benefits such as milk production efficiency. The economic implications are significant, as the Ahmer coat colour's association with superior milk production capabilities likely influences herders' mating choices and herd compositions. The preference for camels with certain coat colours, particularly those believed to yield better milk, is indicative of a broader trend in pastoral economies where genetic traits are selected based on direct economic benefits.

Table 2. Colour traits of dromedary camels (coat, eye, muzzle, eyelid, ear, and foot) in the El Oued region.

Parameter	Modality	Percentage (%)
Coat Colour	Abyad	12.70
	Achgher	9.52
	Adkhan	7.94
	Ahdjel	23.81
	Ahmer	19.05
	Asfer	14.29
	Azram	1.59
	Azreg	9.52
	Zelraf	1.59
Eye Colour	Blue	1.59
	Brown	85.71
	Black	12.70
Muzzle Colour	Non-pigmented	47.62
	Pigmented	52.38
Eyelid Pigmentation	Not Pigmented	92.06
	Pigmented	7.94
Ear Pigmentation	Not Pigmented	76.19
	Pigmented	23.81
Foot Pigmentation	Not Pigmented	61.90
	Pigmented	7.94
	Partially Pigmented	30.16

Four eye colours of camels have been identified in El Oued province (Fig 3). The predominance was seen with brown eyes (85.71%) in camels of present study (Table 2). In contrast, Meghelli *et al* (2020) presented differing results in their study of camel ecotypes, observing that 100% of the Sahraoui ecotype possessed brown eyes, while the Steppe camel ecotype (Naili) exhibited a combination of black (58%) and brown eyes (41.82%). Notably, blue eyes

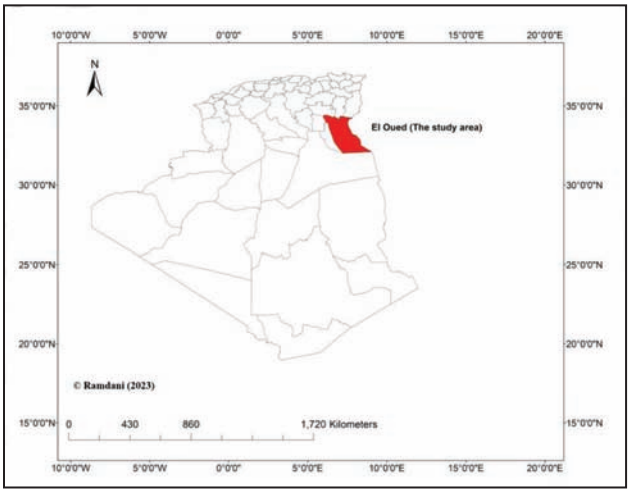


Fig 1. Localisation of the El Oued region (Ramdani, 2021).

were absent in both ecotypes studied by Meghelli *et al* (2020). This discrepancy highlights regional variations in eye colour distribution, potentially influenced by differing genetic backgrounds, environmental pressures, and breeding practices. Furthermore, the presence of blue eyes in the Tergui ecotype with the Zelraf coat pattern corroborates observations by Volpato *et al* (2017), who identified a genetic linkage between coat patterns and eye colour variations in camels. This association suggests that specific genetic markers responsible for the Zelraf (piebald) coat may also influence eye pigmentation, resulting in the rare occurrence of blue eyes within this subgroup.

This study examined three types of pigmentation in dromedary camels, i.e. muzzle (Fig 4), eyelid (Fig 5) and foot (Fig 6) pigmentation. Regarding muzzle pigmentation, there is a notable distribution between pigmented (52.38%) and non-pigmented muzzles (47.62%). These observations suggest that the allele responsible for Abyad Colouration may be linked to non-pigmentation at the muzzle, indicating a possible trait association that could be useful in predicting phenotypic characteristics based on coat colour alone. Conversely, the presence of pigmented muzzles in a substantial proportion (63.6%) of individuals with the Adkhan and Azreg coat colours highlights an important distinction in the genetic traits associated with these Colourations (Table 2).

In contrast, non-pigmented eyelids were observed in the majority of animals (92.06%), while only 7.94% displayed pigmented eyelids (Table 2). These findings differ significantly from the study of Bouzid's (2018), which reported that 94.27% of camels had pigmented eyelids compared to 6.08% with non-pigmented eyelids. The study also identified three types of foot pigmentation. The results indicated that 61.90% of the animals have non-pigmented feet, 7.94% have pigmented feet, and 30.16% possess pigmented front feet. Additionally, pigmentation patterns were found to be related to coat colours. Notably, camels with the Abyad coat colour exhibited no pigmentation, exclusively featuring non-pigmented muzzles. Most camels with pigmented eyelids belong to the Adkhan and Achgher coat colours. Furthermore, a significant majority (86.6%) of animals with the Ahdjel coat colour have pigmented front feet. All camels with Adkhan, Abyad, and Achger coat colours have feet that match their coat colour, while the majority of animals with Azreg, Asfer, and Ahmer coat colours (74%) also exhibit feet that correspond to their coat colour.

In examining camel characteristics, the findings regarding hump size, posture, and position provide significant insights into their health and nutritional status. According to Table 3, small hump size is the most prevalent, accounting for 65.08% of the ecotype, while medium and large sizes were 17.46% each. This distribution indicates a clear correlation between hump size and the fattening condition of the animals; larger humps generally correlate with improved nutritional health. As camels' conditions enhance, their hump size tends to increase, suggesting that hump size serves as a reliable indicator of overall health and fat reserves. Supporting the observations of Bengoumi *et al* (2005), this relationship underscores the critical role of nutrition in camel husbandry. Additionally, the results indicate that all studied animals possess an upright or erect hump, a finding corroborated by Tandoh *et al* (2018). In terms of hump position, data from Table 3 reveal that all animals exhibited a central hump position. However, this finding contrasts with Bouzid's (2018) report, which noted that only 59.56% of the animals had a centrally placed hump, with 14.71% presenting an anterior position and 25.74% a posterior position. Furthermore, all animals in the study exhibited lateral ear orientation. Notably, Tandoh *et al* (2018) also found that all camels have erect humps, aligning with the current study's observations.

Table 3. Distribution of dromedary camels based on hump characteristics in El Oued region.

Parameter	Modality	Percentage (%)
Hump Size	Big	17.46
	Medium	17.46
	Small	65.08
Hump Orientation	Upright	100
Hump Position	Medium	100

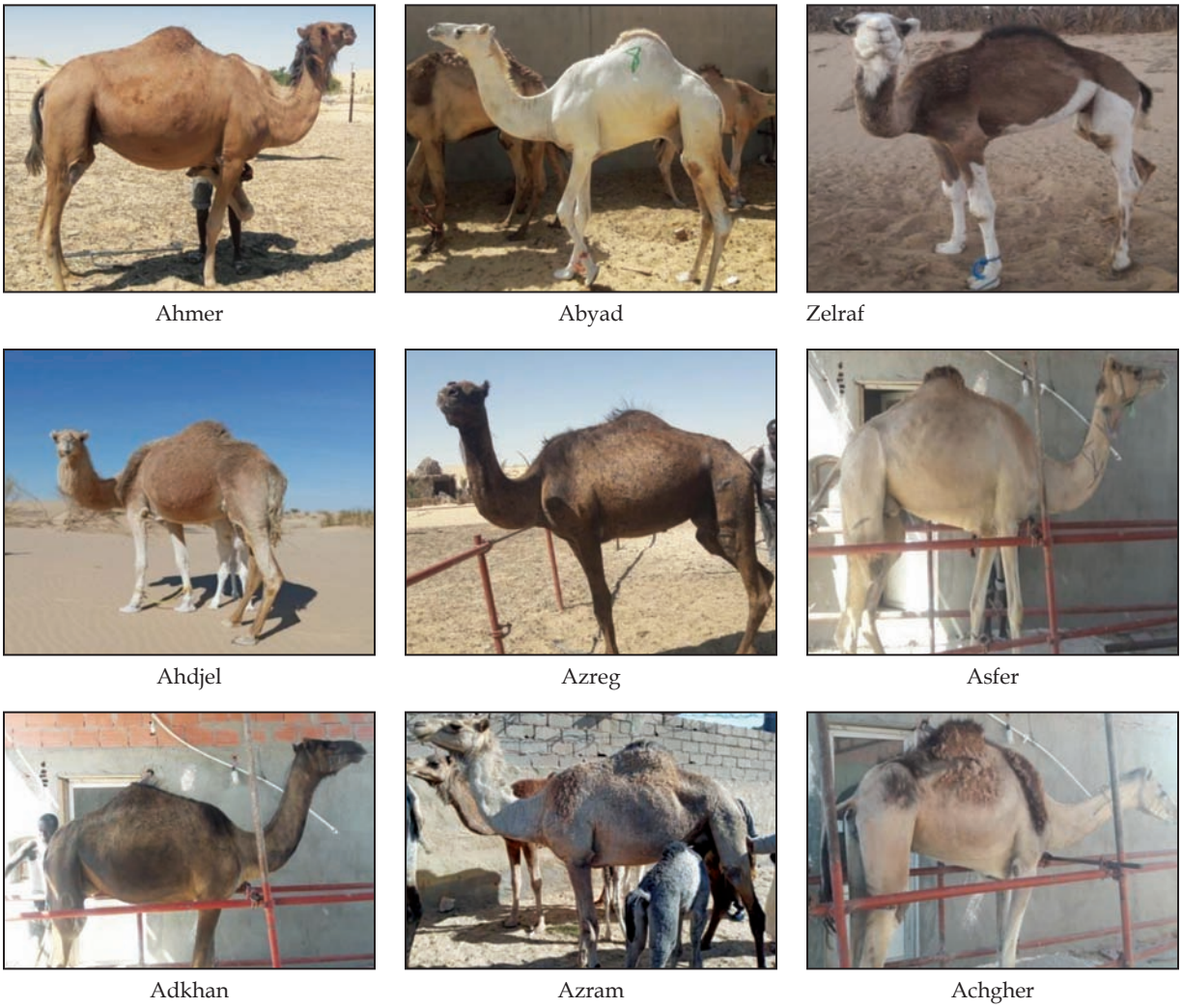


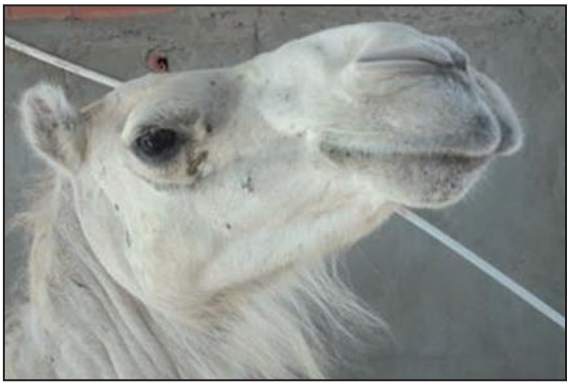
Fig 2. The different coat colours of camels in El Oued region resulting in the rare occurrence of blue eyes within this subgroup.

Fig 3. Eye colour of camel in El Oued region and Ahmer coat colours (74%) also exhibit feet that correspond to their coat colour.

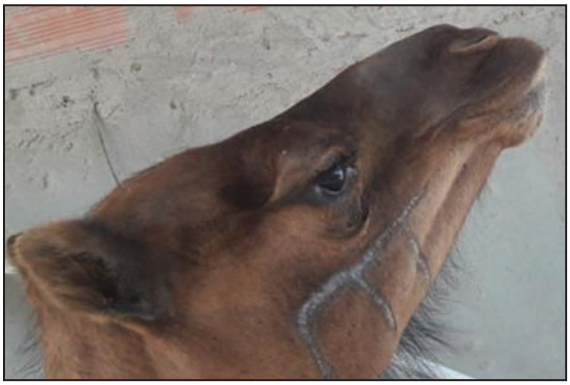
The analysis of the facial profiles of dromedaries reveals intriguing discrepancies compared to previous studies (Table 4). According to Bouzid (2018), the three most common facial profile types are concave, convex, and straight. However, the results presented in Table 4 indicate that all the animals studied possess a straight facial profile (Fig 10), sharply contrasting Bouzid's findings, where only 49.06% had a straight profile, while 28.30% exhibited a concave profile and 22.64% a convex profile. Additionally, the data in Table 4 highlight

Table 4. Facial, backline and ear Profile Observed in Dromedary Camels in El Oued Region.

Parameter	Modality	Percentage (%)
Facial Profile	Straight	100
Backline Profile	Straight	100
Ear Orientation	Lateral	100

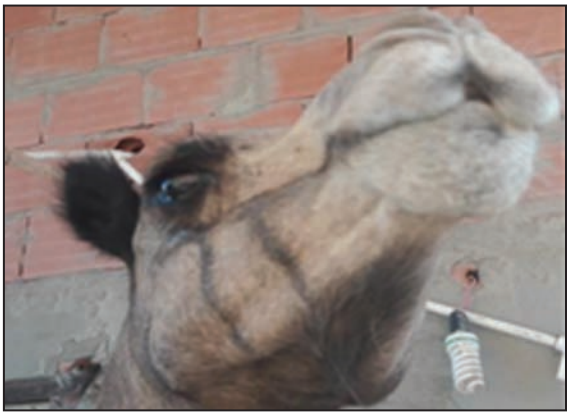


Non-Pigmented Muzzle



Pigmented Muzzle

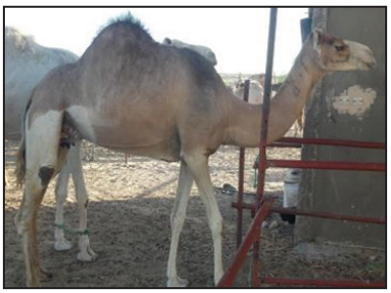
Fig 4. Muzzle Pigmentation in camel.



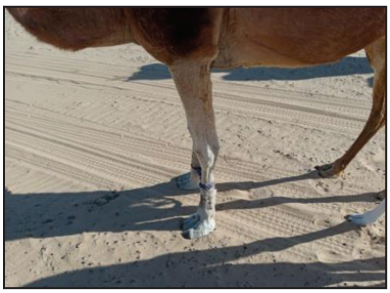
Pigmented Eyelid

Non-Pigmented Eyelid

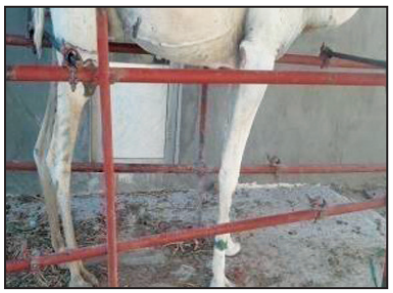
Fig 5. Eyelid pigmentation of camel.



Pigmented Feet



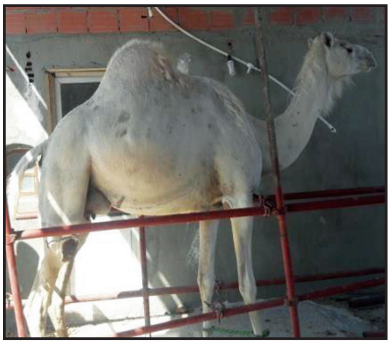
Pigmented Front Feet



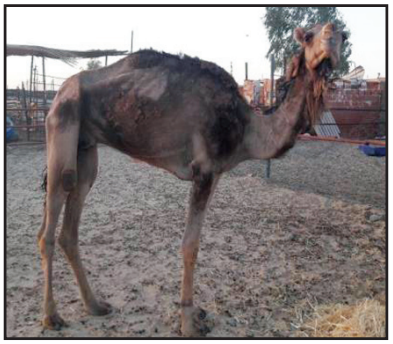
Non-Pigmented Feet

Fig 6. Foot Pigmentation of camel.

Bosse de taille moyenne → Medium-sized hump



Bosse de grande taille → Large-sized hump



Bosse de petite taille → Small-sized hump

Fig 7. Different hump size of camel in El Oued region.

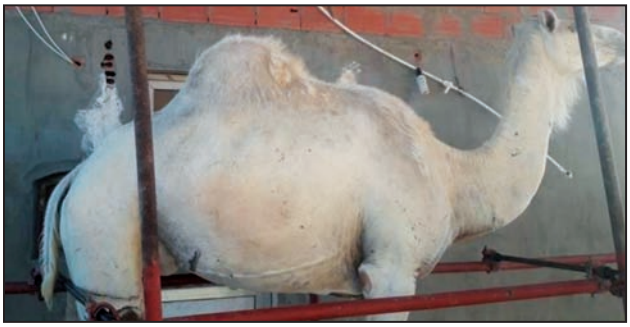


Fig 8. Hump in a central position of a camel.

Fig 9. Lateral orientation of the ear.

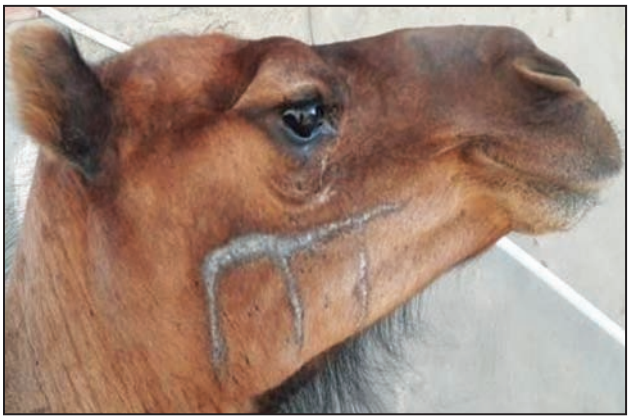


Fig 10. Straight facial profile of a camel's head.

Fig 11. Straight back line of camel.

that all animals also feature a straight back line profile, as illustrated in Fig 11.

This foundational characterisation not only contributes to the understanding of local genetic diversity but also emphasises the importance of preserving and promoting these traits in breeding practices. The results underscore the potential for future research focused on camel breeding strategies and conservation efforts in Saharan Algeria, which are essential for maintaining the genetic integrity and viability of this vital livestock resource. Overall, this study serves as a crucial step towards improving the management and sustainability of dromedary camel ecotypes in the region.

Authors' Contributions:

Maria Chikha contributed to the conception, design, and writing of the manuscript. Safia Tennah supervised the work and provided overall guidance. Aicha Mouane and Nacira Ramdani approved the manuscript for submission. Fahima Neffar contributed to the validation of the study. Safia BEN AMOR: contributed to the validation of the study.

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GROSS AND MICROSCOPIC HEPATIC LESIONS OF DROMEDARY CAMELS SLAUGHTERED IN EASTERN PROVINCE OF KINGDOM OF SAUDI ARABIA

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ABSTRACT

This study was carried out to describe the gross and microscopic hepatic lesions of dromedary camels slaughtered at Al omran abattoir in the Eastern Province of Saudi Arabia. The grossly affected livers from 120 camels with different ages, sexes, and unknown history were collected from freshly slaughtered animals, and examined grossly and microscopically. The total number of liver lesions recorded in this study were 43 (36.66%) cases out of 120 samples of camel livers collected. The pathological changes observed in this study, were degenerative changes and focal hepatic necrosis (10, 8.33%), partial liver cirrhosis (8, 6.66 %), diffuse liver cirrhosis (6, 5 %), liver abscesses (5, 4.16 %), hydatid cysts (4, 3.33 %), haemorrhages and congestion (4, 3.33%), chronic suppurative hepatitis (3, 2.5%) and pigmentation (3, 2.5%). In the present study, degenerative changes, necrosis and liver cirrhosis were the most common hepatic lesions affecting the collected liver samples of camels during the study period. In some liver sections, partially degenerated parasitic ova and hydatid cysts were identified as cause of focal necrosis and calcification.

Key words: Camel, hydatid cyst liver lesions, liver cirrhosis

The camel suffers from many disease conditions including those that affect the kidneys (Taha *et al,* 2007, Barakat *et al,* 2021). Recently there is continued interest concerning incidence of liver diseases in dromedary camels, and various liver diseases have been reported during necropsy examination (Van Saun *et al,* 2000). As in all domestic animals, the liver of camels is considered the most important organ because most of the metabolic activities of the body take place in the liver (Siddig, 2002; Watkins and Seef, 2006; Radostits *et al,* 2007). In general, hepatic dysfunction may be diagnosed by the evaluation of clinical history, physical examination, biochemical tests, hepatic imaging, gross and histopathological examinations (Al-Sobayil, 2008). In many previous studies, researchers have explained several hepatic disorders associated with the liver of camels in many countries, including hepatitis and cirrhosis (El-Mahdi *et al,* 2013; Tharwat, 2020), hydatid cysts (Ahmadi, 2005, Al-Hadi and Saad, 2012), *Fasciola hepatica* (Eslami *et al,* 2003), linguatulosis (Haddadzadeh *et al,* 2009; Oryan *et al,* 2011) and abscesses of *Corynebacterium pseudotuberculosis,* (Hawari, 2008). In addition, neoplasms arising from genetic mutations or environmental carcinogens were reported in different camels tissues (Weiss and Walz, 2009; Simmons and Fitzgerald, 2005). The persent study was aimed to clarify the main gross and microscopic hepatic lesions of dromedary camels slaughtered at Al Omran abattoir in the Eastern region of Saudi Arabia.

Materials and Methods

The grossly affected livers from 120 dromedary camels with different ages and unknown history were collected from freshly slaughtered animals at Al omran abattoir in the Eastern Province of Saudi Arabia and examined carefully for macroscopic lesions. For histopathological study, tissue samples were taken from the hepatic lesions and fixed in 10% neutral buffered formalin. These were processed and embedded in paraffin. Sections of 5 µm thickness were cut and stained with haematoxylin and eosin.

Results

Various gross and microscopic lesions observed in the liver of 120 examined camels are summarised in table 1.

Pathological manifestations

1. Patterns of Focal hepatic Necrosis

Hepatocellular degeneration and focal necrosis and calcification were the most common hepatic lesions seen in this study. The lesions were in the

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form of round white or gray spots or areas 4-6 cm in diameter, distributed under the liver capsule or within the liver tissue (Figs 1a, 1b).

Microscopically, these appeared as a coagulative type of necrosis at the centre of the lesion surrounded by cellular infiltrations composed of macrophages, tissue cells, lymphocytes, plasma cells, and then granulation tissue (blood capillaries and fibroblasts) (Fig 2).

Table 1. Pathological changes observed in the liver of 120 examined camels.

Pathological changes	Number of samples	Percentage
Focal hepatic necrosis	10	8.33
Partial liver cirrhosis	8	6.66
Diffuse liver cirrhosis	6	5.00
Liver abscesses	5	4.16
Hydatid cysts	4	3.33
Haemorrhages and congestion	4	3.33
Chronic suppurative hepatitis	3	2.50
Pigmentation	3	2.50
Total	43	36.66

2. Partial and diffuse liver cirrhosis

Liver cirrhosis appeared on partial or diffuse liver fibrosis. The affected parts were hardened and white in colour. Some cases were associated with peritoneal ascites. However, parasitological examination of these camels was negative. Microscopic examination revealed fibrosis characterised by proliferation of fibroblasts and collagen fibres in the liver capsule, around the liver lobules, or around the hepatocytes (Fig 3). In advanced cases, fibrosis involved many liver lobules (diffuse fibrosis) or large parts of the liver, where the affected parts were composed of connective tissue containing remnants of atrophic hepatocytes (Figs 4 and 5).

3. Liver abscesses

Grossly, the lesions were characterised by the presence of a purulent material surrounded by fibrous tissue, usually protruding from the liver surface. The cross-section revealed a purulent material that was white or greenish in colour and found calcified (Figs 6 and 7). Microscopically, the lesion showed a liquefied necrosis surrounded by numerous neutrophils and a fibrous capsule (Fig 8).

4. Hydatid cysts

The lesions were characterised by the presence of variable numbers of prominent vesicles on the surface or within the liver tissue (Fig 9). Microscopically, the lesion showed the presence of a fibrous outer membrane, hepatocyte atrophy, and cellular infiltration (Fig 10).

5. Liver haemorrhages and congestion

The affected liver was characterised by its enlargement and change in colour to crimson or dark red, with the presence of haemorrhagic spots, clear liver lobules (Figs 15) and blood flow when the liver was cut. Microscopic examination showed that there was congestion in the blood sinuses in the centres of the lobules (Fig 16), although in some cases it included most of the lobules, as it was accompanied by atrophy and necrosis of the hepatic cells, and the lobules were transformed into blood fields containing some atrophied hepatic cells

6. Chronic suppurative hepatitis

This lesion was characterised by hepatomegaly and the presence of multiple, large, irregular purulent spaces with a fibrous capsule dividing the lesions into what looked like honeycombs. The lesions were usually calcified and accompanied by peritoneal oedema. (Figs 13 and 14).

7. Pigmentation

Irregular black spots were observed on the surface of the liver and were found to be spread within the visceral tissue on cut sections (Fig 11). Microscopiclly, melanocytes were seen in the liver capsule in the connective tissue surrounding the liver lobules (Fig 12).

Discussion

An overall occurrence of pathological lesions affecting the liver of camels examined in the present study was recorded as 36.66%. However, other researchers found higher occurrence in dromedary camels in other countries i.e., Al-Hadi and Saad (2012) and Hamza *et al* (2017) in Sudan, Nourani and Salimi (2013) in Iran and Asopa *et al* (2022) in India.

Histopathological examination performed in fresh animal tissues is an extremely useful diagnostic method and can often detect various hepatic diseases as infectious, toxic, or obstructive (Al-Sobayil, 2008). In the present study, hepatocellular degeneration and necrosis, were seen in (8.33%) 10, liver samples. However, higher incidence of degenerations and necrotic changes were reported by Salem and Azza (2011), Borai *et al* (2013), Zakian *et al* (2016) and Tavella *et al* (2018). The occurrence of partial and diffuse liver cirrhosis, was reported in the present

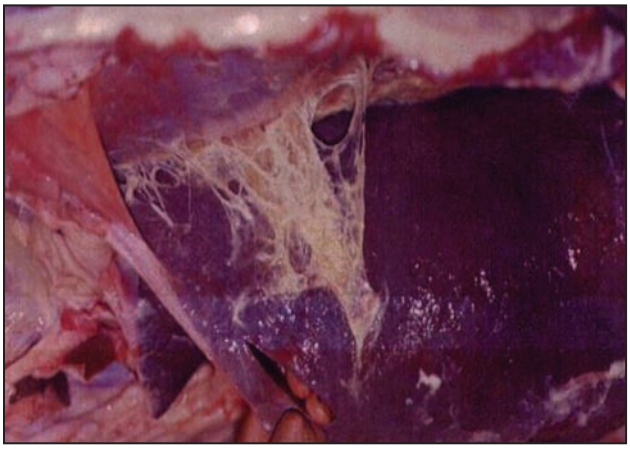

Fig 1a. Fibrinous adhesions on the liver capsule. Notice marked fibrin deposition along the capsule surface.

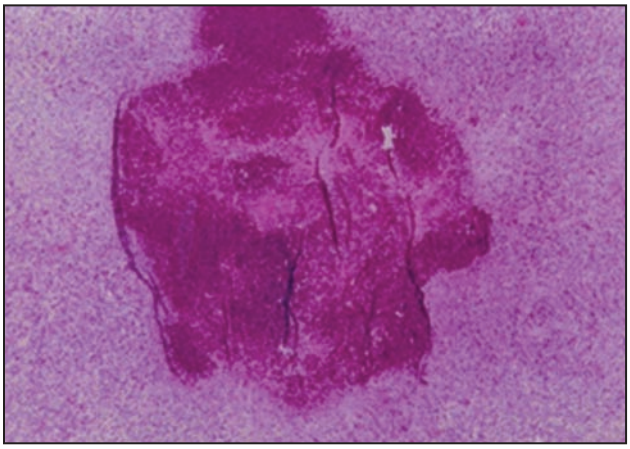

Fig 1b. Note the areas of coagulative necrosis of the hepatic cells surrounded by granulation tissue.

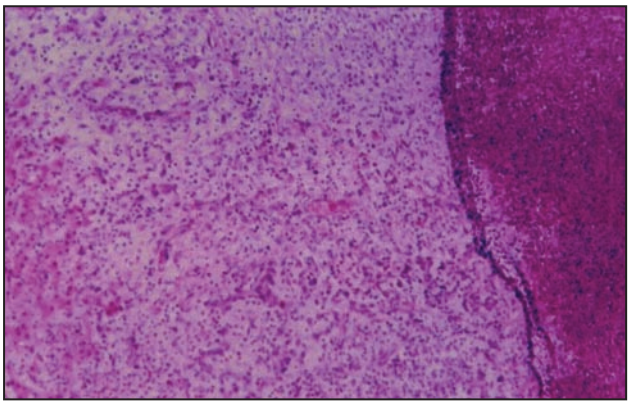

Fig 2. Localised liver coagulative necrosis 100 ×.

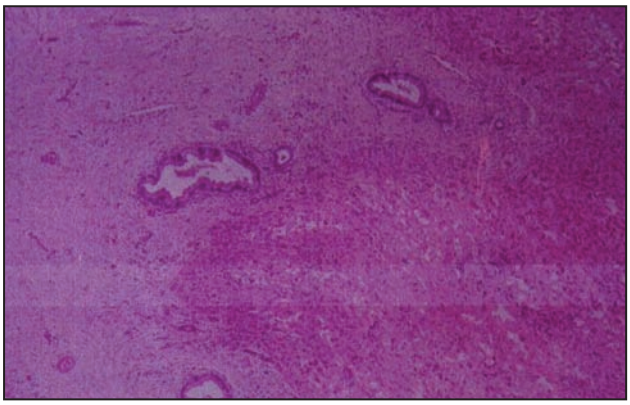

Fig 3. Partial liver cirrhosis proliferation of fibrous tissue around the hepatic lobules with the continued presence of bile ducts 100 x.

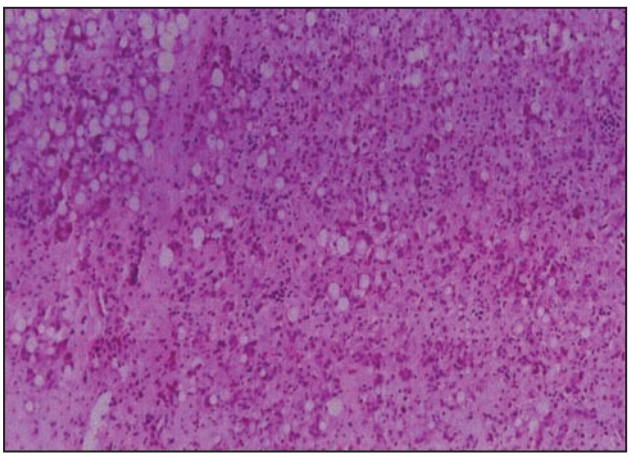

Fig 4. Diffuse liver cirrhosis: Fibrous tissue proliferation with hepatocellular atrophy 100 x.

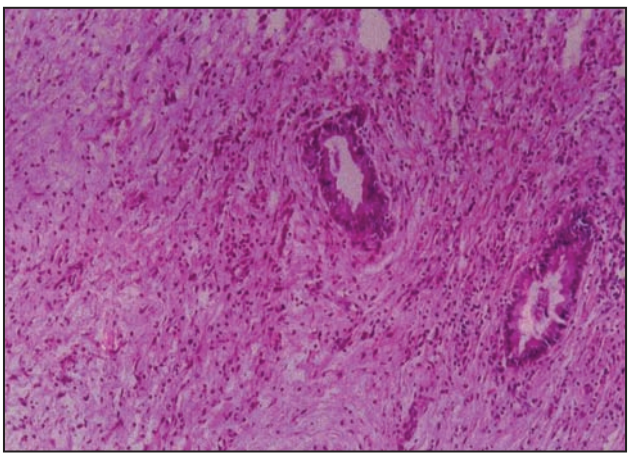

Fig 5. Proliferation of fibrous tissue and the disappearance of hepatic cells, with the presence of the bile ducts 200 ×.

study. However, higher incidence was observed by Jamshidi and Zahedi (2014). The occurrence of liver fibrosis is frequently associated with parasitic infestations (Singh, 1998 and Ibrahim *et al,* 2021) and it may occur as a result of certain environmental pollutants, such as copper, (Jung and Thornburg, 1989), arsenic and selenium, (Groom *et al,* 1995) and diazinon (Agab, 2003).

Previous reports conducted in Sudan, Egypt and Kenya have shown that some microbes have been

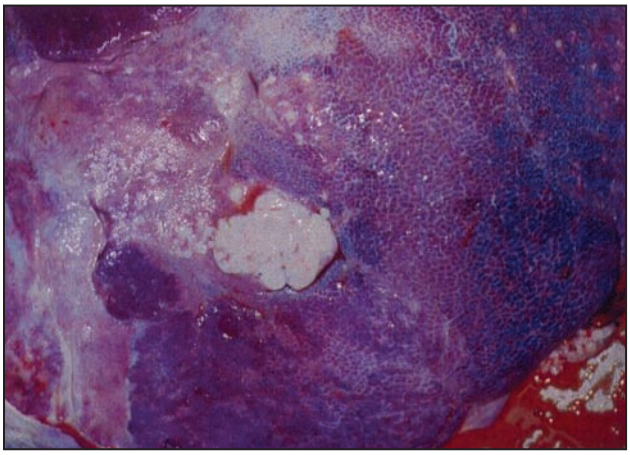

Fig 6. Liver abscesses. Note the presence of a liver abscess protruding on the surface of the liver.

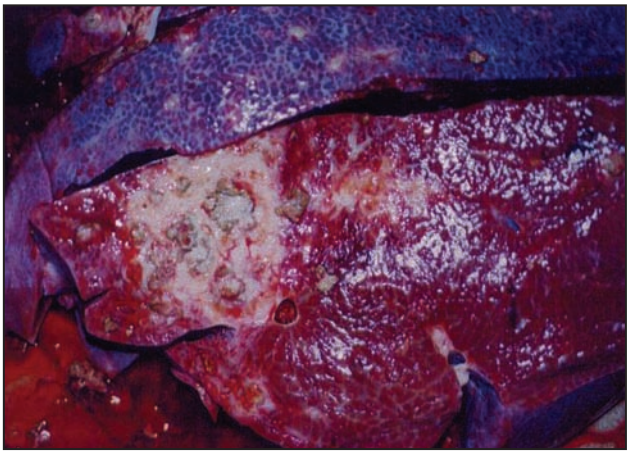

Fig 7. Cross section of an abscess consisting of purulent fluid surrounded by fibrous tissue.

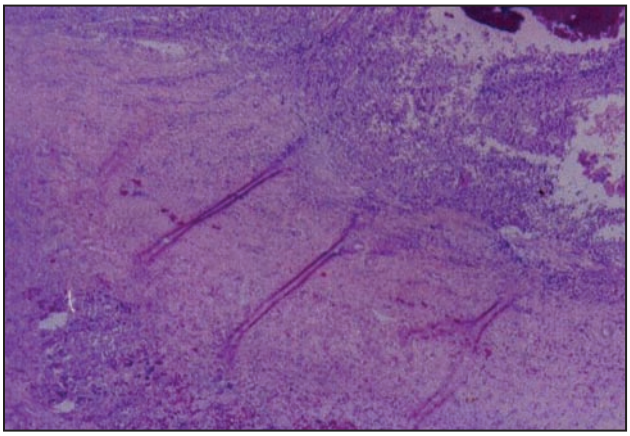

Fig 8. Liver abscess. Liquefied necrosis surrounded by inflammatory cells and fibrous tissue 40 ×.

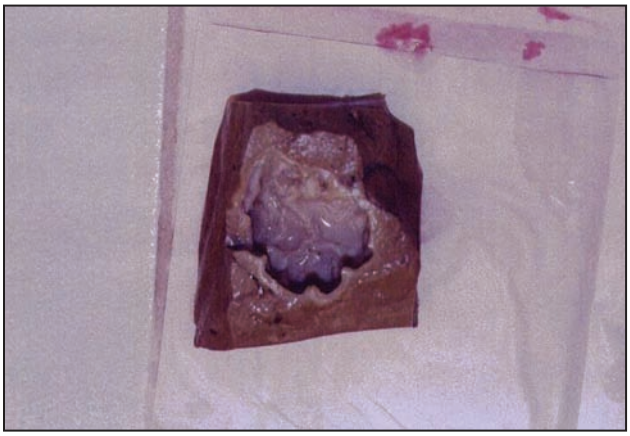

Fig 9. Hydatid cysts. Note the presence of a thick fibrous outer membrane and cysts imbedded in the liver tissue.

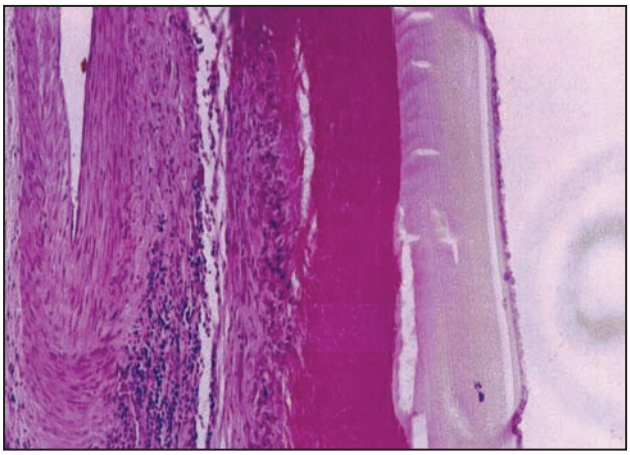

Fig 10. Hydatid vesicles. Note the presence of a fibrous outer membrane, hepatocyte atrophy, and cellular infiltration 100 X.

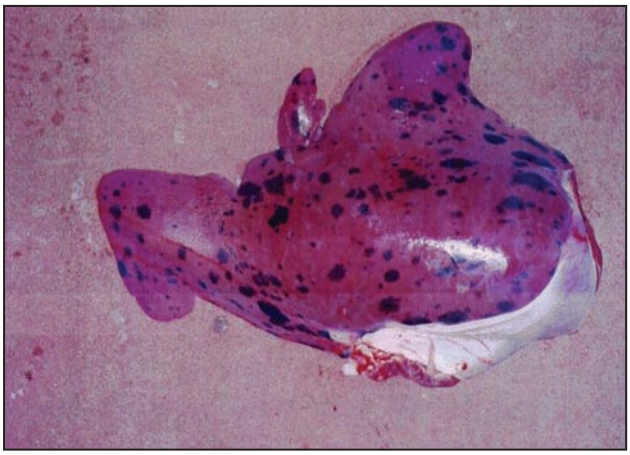

Fig 11. Liver melanosis. Note the presence of black areas on the surface of the liver.

associated with liver necrosis, cirrhosis and hepatitis in dromedary camels (Hennessy and Porth, 2004, Thapa and Walia, 2007; Ayman, 2008).

The occurrence of liver abscesses was found low in animals of present study. A higher incidence was reported by Elhadi and Saad (2012), (9%), and Aljameel *et al* (2014) (13.5%) in Sudan. Whereas a lower incidence was recorded in Jordan (1.2%) (Al-Ani *et al,* 1998) and in Iran (0.64%) (Nourani and Salimi, 2013). The occurrence of liver hydatid

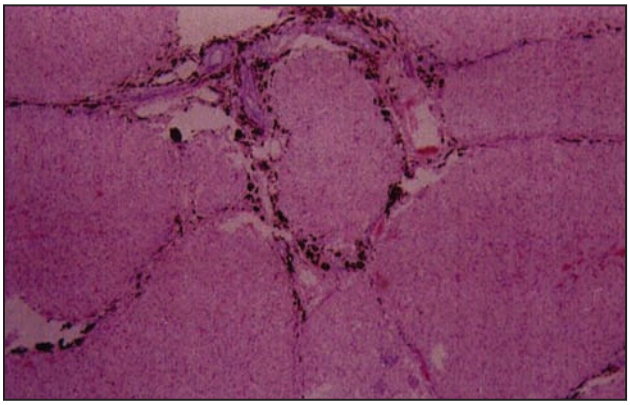

Fig 12. Liver melanosis. the presence of black areas on the surface of the liver.

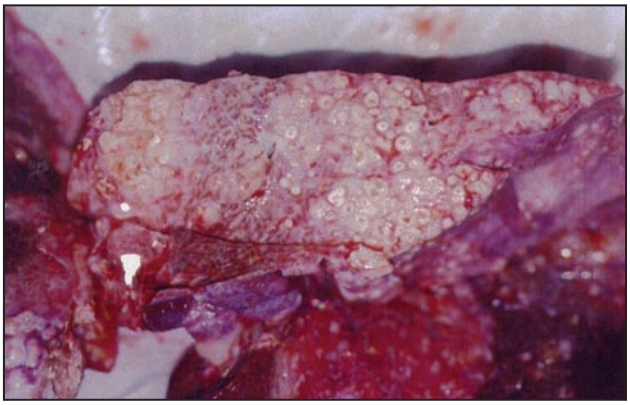

Fig 13. Chronic purulent hepatitis, Cross section of the liver showing the appearance of the lesion as multiple purulent lesions surrounded by fibrous tissue.

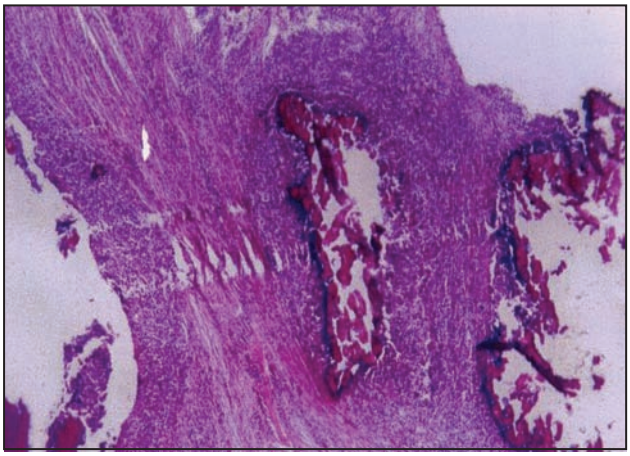

Fig 14. Chronic purulent inflammation. Note the calcification of the purulent lesions, surrounded by inflammatory cells and a fibrous capsule. 40 X.

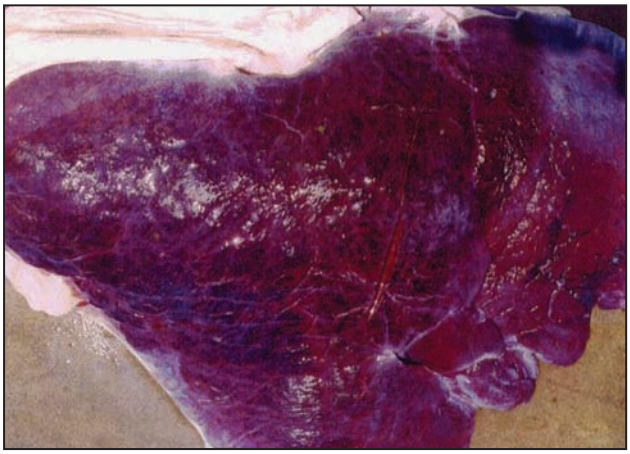

Fig 15. Liver Congestion Note the enlarged liver with dark spots under the surface of the liver capsule.

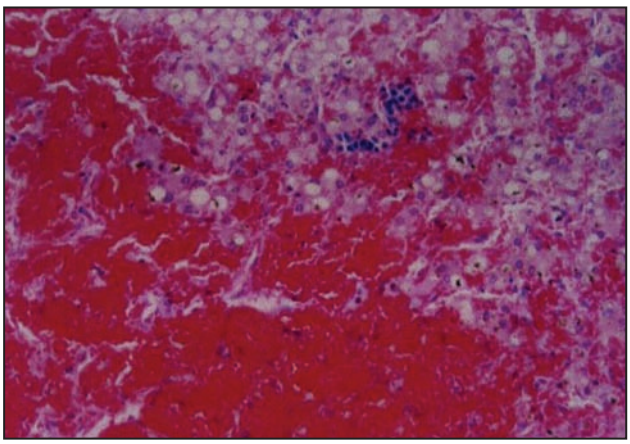

Fig 16. Liver congestion. Note the presence of partial haemorrhage with hepatic cell necrosis.

cysts as noted in present study was also reported in camels by Mirazaei *et al* (2016) in Iran. However, higher incidence of liver hydatatosis was reported in Sudan (Omer *et al,* 2010). The incidence of hydatid liver disease in man and animal is very common worldwide and frequently associated with the *E. granulosus* infection (Belina *et al,* 2011).

The occurrence of liver haemorrhages and congestion as observed in present study was in partial agreement with the observations of Singh (1998) and Ibrahim (2021). Chronic suppurative hepatitis lower than those reported by previous investigators in Jordan (Al-Ani *et al,* 1998) and in Iran (Nourani and Salimi, 2013). Purulent inflammatory conditions in camel liver could be attributed to a number of etiologies, the most important of which are bacterial infections, environmental changes and husbandry practices. (Hawari, 2008; Hegazy *et al,* 2010).

Irregular black spots of melanin noted on the surface of the liver is generally considered a benign condition, often found incidentally during slaughter. Naturally occurring black pigmentation of the camel liver, often with concurrent lung and kidney discolouration was previously reported in Iran (Gholam *et al,* 2014) and in Australia (Meat and Livestock Australia, 2018). Affected camels of present

study appeared healthy and it was considered is observed as an incidental finding at slaughter.

In conclusion, losses of camel livers in slaughterhouse are economically significant. Therefore, routine examination of camels and early diagnosis, of hepatic disorders, using modern diagnostic techniques should be considered on priority as it affect health of human and camel both.

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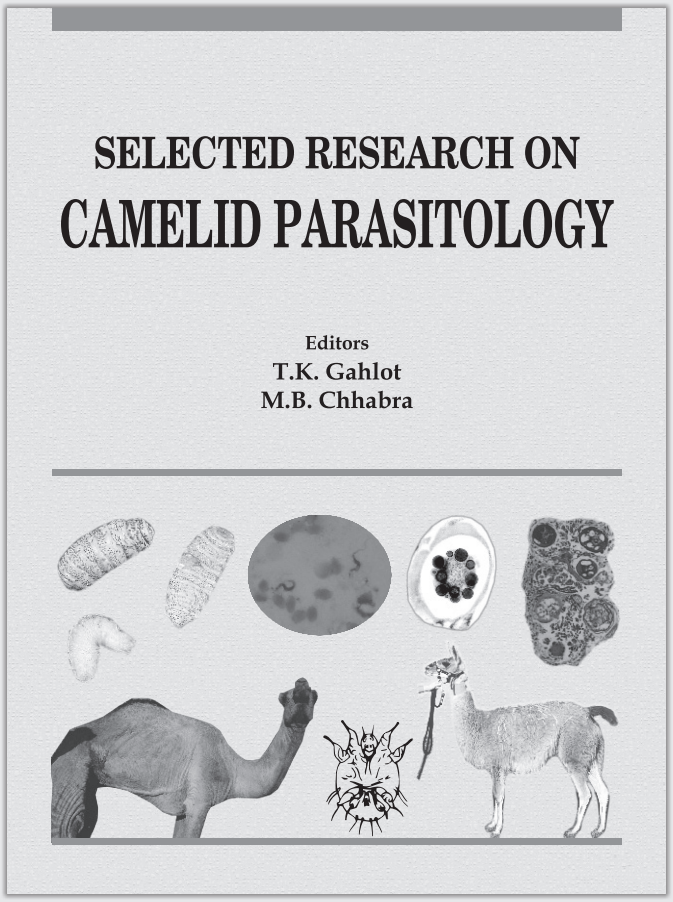
SELECTED RESEARCH ON
CAMELID PARASITOLOGY

Hard bound, 291 pages, few figures coloured

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



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ncRNAs REGULATION OF SALT AND DROUGHT RESISTANCE IN CAMELS

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ABSTRACT

Camels exhibit extraordinary resilience to arid environments by enduring extreme salinity and drought through specialised adaptations. Emerging research highlights the critical regulatory roles of non-coding RNAs (ncRNAs) in mediating salt and drought resistance. ncRNAs play a pivotal role by integrating osmoregulatory, metabolic and antioxidative responses to fortify camels against extreme desert condition. This review synthesises recent advances in our understanding of evolutionary adaptations and proposes strategies for improving stress tolerance in agriculturally significant species.

Key words: Camels, drought resistance, homeostasis, metabolic adaptation, ncRNAs

Camels breeding has a long history and has played a pivotal role in sustaining desert and semi-desert ecosystems. These animals have evolved numerous adaptive traits supporting resistance to heat, salinity and aridity environments (Rehan and Qureshi, 2006). As species long habituated to desert environments, camels exhibit stress -resistance mechanisms that enable survival under conditions of water scarcity and elevated temperatures. Comparative genomic studies across desert mammals reveal substantial functional overlap in gene classes and metabolic pathways, reflecting the phenotypic complexity required for adaptation to resource-limited and thermally extreme habitats (Rocha *et al,* 2021).

Non-coding RNA (ncRNA) is commonly employed for RNA transcript that does not encode proteins, high-throughput techniques have produced remarkable evidences for ncRNA-associated interactions in different kinds of cellular functions (Eddy, 2001). It indirectly controls a wide range of biological processes, including cellular metabolism, developmental programmes, transcriptional activity, post- transcriptional modifications, mRNA stability and translation and even protein degradation and translocation (Storz, 2002; Mattick *et al,* 2006). The post -transcriptional regulatory capacity of ncRNAs significantly impacts gene expression dynamics. Studies have demonstrated that miRNA can inhibit the expression of protein-coding genes by binding to 3′ untranslated regions (UTRs) and protein-coding regions of a targeted mRNA (Bartel, 2004). The ncRNA family encompasses multiple subtypes: small interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), tRNA-derived small RNAs (tsRNAs) and others (Borsani *et al,* 2005; Girard *et al,* 2006; Ning and Li, 2018). Small interfering RNAs (siRNAs) constitute a class of 20-25 nucleotide double-stranded RNA molecules originating from perfectly complementary fold-back structures (Hamilton and Baulcombe, 1999). MicroRNAs (miRNAs), endogenous small ncRNAs of approximately 21-23 nucleotides, are processed from transcribed hairpin precursors (Lee *et al,* 1993; Lagos-Quintana *et al,* 2001). Piwi-interacting RNAs (piRNAs) derive their nomenclature from associated PIWI proteins, as these Argonaute family members mediate precursor piRNA processing through the Ping-Pong amplification cycle (Aravin *et al,* 2007; Siomi *et al,* 2011). Long non-coding RNAs (lncRNAs), defined as non-protein-coding transcripts exceeding 200 nucleotides, were initially identified in murine systems through early 1990s cDNA library analysis (Brannan *et al,* 1990). long ncRNAs were first described during the large-scale sequencing of full-length cDNA libraries in the mouse (Okazaki, 2002) (Table 1).

The first draft of both domestic (*Camelus bactrianus*) and wild Bactrian camel (*Camelus ferus*) genomes was reported in 2012. The size of Bactrian camel genome was reported as 2.38 Gb and contained 20,821 genes (BCFSAC, 2012). A similar report, related to sequencing the genome of Bactrian, dromedary and alpaca camels (*Vicugna pacos*), was published in 2014. The genome size of Bactrian camel reported

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in this study (2.45 Gb) was similar to the earlier reported. The non-coding RNA genes of Dromedary, Bactrian and alpaca genomes shared similar copy numbers. non-coding RNA genes showed remarkable consistency: 1,942 in Bactrian camels, 2,209 in dromedaries and 2,328 in alpacas (Wu *et al,* 2014).

Camels possess multiple physiological adaptations to survive harsh environments, including: water conservation mechanisms, highly efficient kidneys, specialised fat storage in humps, dehydration-resistant blood cells and metabolic flexibility. These adaptations are supported by specific genetic foundations: Water conservation primarily involves genes regulating renal function, particularly those associated with ion transport and urine concentration mechanisms. Additional genetic adaptations likely minimise water loss through reduced sweating and respiratory evaporation. Metabolic adaptations feature two key components: First, the preferential utilisation of fat stores (rather than carbohydrates) to generate metabolic water during prolonged fasting. Second, enhanced antioxidant systems combat oxidative stress induced by dehydration, which could otherwise cause cellular damage (Wu *et al,* 2014). Non-coding RNAs (ncRNAs) serve as master regulators in camels, enabling them to survive extreme aridity and salinity by coordinating salt tolerance and drought resistance through shared and distinct molecular pathways (Fig 1).

Osmoregulation and Ion Homeostasis

Under osmotic stress, ncRNAs modulate osmoregulation and ion homeostasis by targeting ion transport-related genes, such as sodium-potassium pumps and channels, thereby reducing cellular ion toxicity. Notably, sodium reabsorption and water balance in the kidney have been identified as an adaptation to desert environment (Wu *et al,* 2014; Okazaki *et al,* 2002) Hypertonicity serves as the physiological foundation for renal water balance and reabsorption, mediated through a gene network that coordinates water reabsorption with glucose-regulated osmoregulation and water conservation. Specifically, the expression of osmoregulation-associated genes in osmoregulation in the renal medulla (Wu *et al,* 2014).

Aquaporins (AQPs), a family of water channel proteins, play crucial roles in renal water handling and therefore in the regulation of body water homeostasis (Nielsen *et al,* 1999; Ingelfinger *et al,* 2015; Kortenoeven and Fenton, 2014). AQP1, AQP2 and AQP3 were the top three differentially expressed genes in the renal cortex and medulla under water-restricted conditions. This differential expression pattern may enable camels to enhance water reabsorption efficiency in water-scarce environments (Wu *et al,* 2014,). Notably, two AQP2-targeting miRNAs (miR-32 and miR-137) have been shown to downregulate AQP2 expression in kidney collecting duct cells through mechanisms independent of vasopressin regulation (Kim *et al,* 2015; Gomes *et al,* 2018).

The microRNA-30 (miR-30) family, an important subgroup of miRNAs, comprises five precursor members that generate six mature miRNA molecules: miR-30a, miR-30b, miR-30c-1, miR-30c-2, miR-30d and miR-30e. These molecules are encoded by six distinct genes located on human chromosomes 1, 6 and 8 (Mao *et al,* 2018). Research has demonstrated that miR-30 negatively regulates the uPAR-ITGB3 axis through the calcineurin-NFATC signaling pathway, revealing a novel mechanism underlying podocyte injury in glomerular diseases. This discovery elucidates the functional relationships among key molecular players governing podocyte pathophysiology (Lang *et al,* 2019).

lncRNAs play multifaceted roles in osmoregulation and stress adaptation through

Table 1. Classes of regulatory ncRNAs and their sizes and functions.

Name	Definition	Function	Size	Ref
lncRNA	long noncoding RNA	autonomously transcribed RNA that does not encode a protein; often capped and polyadenylated; can be nuclear, cytoplasmic or both	>200 nt	Brannan *et al,* 1990; Okazaki, 2002
miRNA	microRNA	RNA that in complex with AGO protein, uses seed sequences near its 50 end to base pair with a target mRNA to induce deadenylation and decay or translational regulation	22 nt	Lee *et al,* 1993
ncRNA	noncoding RNA	an RNA that does not encode a protein,but has other cellular functions	–	Eddy *et al,* 2001
piRNA	PIWI-associated RNA	RNA that directs the modification of chromatin to repress transcription; best characterised in the male germline	27 nt	Aravin *et al,* 2006; Girard *et al,* 2006

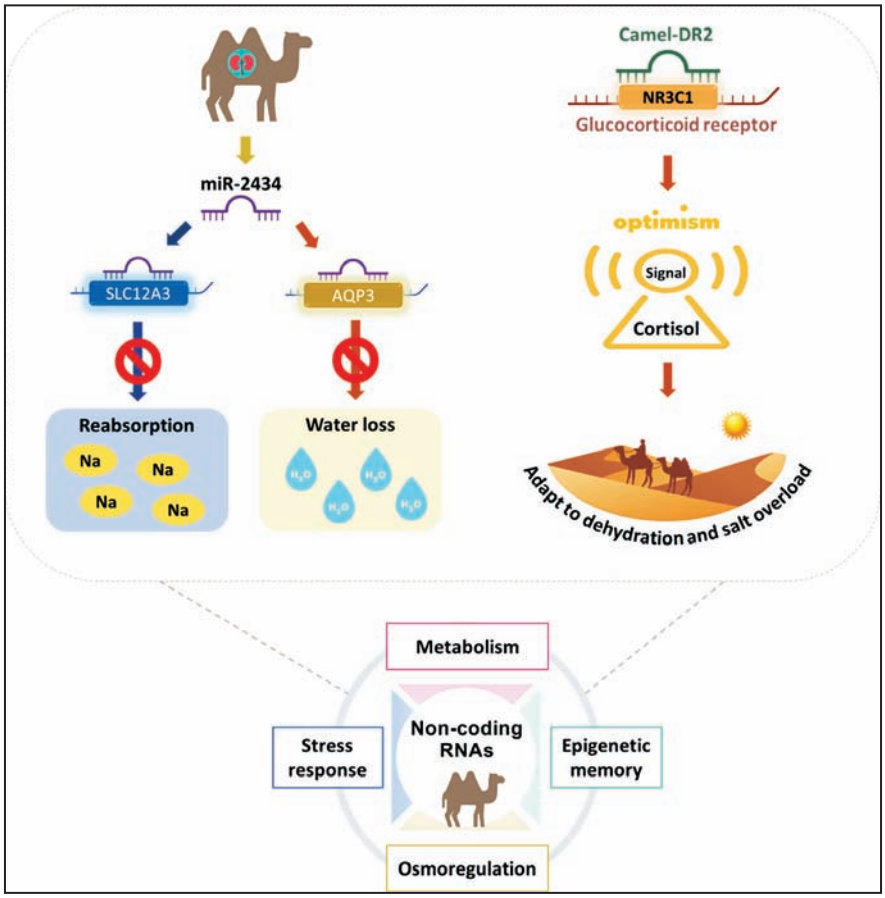

Fig 1. ncRNAs regulate salt and drought stress resistance in camels through various regulatory mechanisms.

diverse molecular mechanisms. Specifically, lncRNAs can serve as molecular scaffolds to stabilise key enzymes such as betaine-aldehyde dehydrogenase (BADH), a rate-limiting enzyme critical for betaine biosynthesis during osmotic stress. The camel-specific lncRNA DR1 exemplifies this regulatory capacity by coordinately modulating two essential pathways: 1) PPARγ-mediated lipid metabolism required for metabolic water generation and 2) SLC26A3-dependent intestinal ion transport (Makishima, 2005), This dual regulation establishes a crucial link between cellular energy production and salt-water homeostasis in dehydrated camels. Another evolutionarily conserved lncRNA, H19, demonstrates complementary protective functions (Wu and Huang, 2023). It stabilises BADH protein levels to maintain betaine synthesis while concurrently upregulating HSP70 expression, thereby preserving proteostasis under dehydration conditions. Furthermore, emerging evidence suggests (Makishima, 2005) that lncRNAs may orchestrate epigenetic responses to environmental challenges through recruitment of chromatin modifiers. This mechanism potentially maintains transcriptionally active chromatin states at stress-responsive loci, including those encoding heat shock proteins and drought tolerance factors.

lncRNAs could stabilise transcripts of urea transporters (e.g., UT-A1/2), enhancing urea recycling to concentrate urine without water loss. Post-transcriptional regulation of ncRNAs in renal cortex, For camel renal cortex under salt stress and water deprivation stress, the diferentially expressed lncRNA.Salt resistance-related genes with significantly different expression were involved in 13 genes, four novel significantly down-regulated lncRNAs (LNC002600, LNC000062, LNC001899 and LNC000331) were detected under salt stress, four novel salt-resistance-related lncRNAs in renal cortex and proposed the ncRNAs-related post-transcriptional regulation pathway to explain how camels respond to salt stress and water-deprivation stress(Cao *et al*, 2019). The response pathway of post-transcriptional regulation concerning salt and water-deprivation stresses was put forward, involving preventing sodium from entering the cell, purifying of water and compensating neutral amino acids by

miR-193b, miR-542-5p interaction with SLC6A19 mRNA.

Under water-deprivation stress, by RNA-sequencing of camel renal medulla associated with regulating water metabolism, 575 significantly differential alternative splicing events (ASEs) along with 17 mRNAs, 26 miRNAs and no lncRNA were detected (Zhang *et al,* 2020). Among these, The down-regulated ACLY and LOC105061856, along with up-regulated PCBP2 and miR-195 potentially targeting LOC105061856 and PCBP2 mRNA were selected as candidate resistance-related genes. Three potential regulatory mechanisms were proposed: (1) suppressed cell dehydration mediated by ACLY downregulation, (2) inhibited aerobic respiration through miR-195-targeted suppression of LOC105061856 and (3) enhanced antioxidative capacity via PCBP2 upregulation despite miR-195's targeting effect. These coordinated adaptations may collectively constitute the molecular basis for camel renal medulla's remarkable water-deprivation tolerance (Zhang *et al,* 2020).

Two copies of CYP2E (CYP2E1 and CYP2E2) , along with CYP2J can help to transform arachidonic acid into19(S)-HETE, whereas CYP4F and CYP4A help to transform it into 20-HETE. Notably, 19(S)-HETE acts as a potent vasodilator in renal preglomerular microvessels and has been shown to promote water reabsorption through tubular mechanisms (Carroll *et al,* 1996; Saadeldin *et al,* 2020).

Dietary salt intake is closely linked to human health, with excessive sodium consumption being associated with increased risks of stroke and cardiovascular diseases. Investigations into the renal medulla of salt-tolerant camels may reveal critical mechanisms underlying high salinity resistance. Through fluorescence in situ hybridisation and dual-luciferase reporter assays, we demonstrated that the long non-coding RNA LNC003834 binds to miRNA-34a, thereby alleviating miRNA-34a-mediated suppression of SLC14A1 mRNA - a transcript encoding a salt absorption inhibitor. These findings suggest that the LNC003834 - miRNA - 34a - SLC14A1 axis functions as a competing endogenous RNA (ceRNA) network (SLC14A1 mRNA, LNC003834 and miRNA-34a) and antioxidant genes (SLC6A1, PCBP2 and PEX5L) (Zhang *et al,* 2020).

Metabolic Adaptation

Metabolic adaptation is mediated through ncRNAs that orchestrate lipid and carbohydrate metabolic reprogramming, thereby optimising energy efficiency under nutrient deprivation. These regulatory molecules can simultaneously inhibit glycolytic pathways while promoting β-oxidation, enabling organisms to maintain energy homeostasis while conserving water reserves. Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors of the nuclear receptor super family and regulate the expression of specific target genes such as those involved in energy and lipid metabolism (Makishima, 2005). Specifically, PPARα is highly expressed in the liver, brown adipose tissue (BAT), heart, skeletal muscle and kidney, which are the tissues that have high capacity for fatty acid oxidation (Takada and Makishima, 2020). lncRNAs and miRNAs activates PPARα/γ to drive fatty acid oxidation in the hump, producing metabolic water during drought while supplying energy for ion-pumping during salt stress. may regulate genes like PPARγ to enhance fat storage in the hump. This fat, when metabolized, produces metabolic water, crucial during droughts.

miR-33, an important regulator of lipid metabolism, target genes involved in metabolism and resulted in improved mitochondrial function and reduced oxidative stress. The reduction in lipid accumulation and liver injury resulted in decreased YAP/TAZ pathway activation, which may be involved in the reduced hepatocellular carcinoma (HCC) progression in livers (Fernández-Tussy *et al,* 2024). It Inhibits SREBP1 (sterol regulatory element binding protein 1) and its downstream lipogenic enzymes in HCC cells via c-Myc. Moreover, SREBP1 is crucial for ACSL4-mediated regulation of lipogenesis as well as HCC cell proliferation and metastasis, This metabolic reprogramming appears to redirect cellular energy resources toward maintaining osmotic equilibrium (Chen *et al,* 2021).

lncRNA UT-AS1 stabilises UT-A1/2 transcripts in renal tissue, enabling urea recycling to concentrate urine (water conservation) and maintain nitrogen balance under high salt intake. the activity of genes in cytochrome P450 (CYP) family are involved in the metabolism of arachidonic acids (KEGG pathway accession code 00590). CYP2J2 is regulated by high-salt diet and its suppression can lead to high blood pressure. Camels are known to be able to take in a large amount of salt apparently without developing hypertension, perhaps because they have more copies of CYP2J genes (Zhao *et al,* 2003).

Evidence from dN/dS-based tests and gene family evolution revealed complex features of adaptation in both Bactrian (*Camelus bactrianus*) and

Dromedary camels (*Camelus dromedarius*), including strong selection in genes from the insulin-signaling pathway regulation, lipid and water metabolism, stress responses to heat, UV radiation and airborne dust (Rocha *et al,* 2021).

AMPK is a critical cellular energy sensor that mainly functions as a metabolic checkpoint to restore energy balance under various metabolic stress conditions (Hardie *et al,* 2012). AMPK is activated by phosphorylation, which maintains cellular energy balance, redox homeostasis and cell survival by regulating glycolysis, fatty acid metabolism, antioxidant reactions and other processes (Lin and Hardie, 2017; Herzig and Shaw, 2018). Liu *et al* (2016) proposed a feed-forward model of NBR2-AMPK regulation, in which the lncRNA NBR2 was induced by the liver kinase B1 (LKB1)-AMPK pathway under energy stress and in turn NBR2 interacted with AMPK and promoted its phosphorylation.

The physiological experiments demonstrated that the elevated blood glucose in camels may be caused by their strong capacity for insulin resistance (Guo *et al,* 2021). Consistent with this argument, numerous rapidly evolving camel genes are functionally associated with both Type II diabetes mellitus (KEGG:04930) and the insulin signaling pathway (KEGG:04910). Specifically, insulin (INS) binding to its receptors induces tyrosine phosphorylation of insulin receptor substrates (IRSs). This phosphorylation cascade subsequently activates PI3K and AKT kinases, initiating downstream processes that facilitate glucose uptake followed by storage (Muoio and Newgard, 2008; Turewicz *et al,* 2025).

Oxidative Stress Adaptability

Oxidative stress resilience is achieved through ncRNA-mediated regulation of antioxidant defenses. High salt intake triggers oxidative damage by disrupting this balance. miRNAs may upregulate antioxidant enzymes by suppressing inhibitors like KEAP1 (which represses Nrf2). Genes encoding antioxidative transcription factors, including Nrf2 (Wang *et al,* 2016), heat shock factor-1, activator protein-1 complex, p53, nuclear factor-kB and signal transducer and activator of transcription 4 exhibited upregulation in the water-restricted renal medulla. This transcriptional reprogramming was complemented by the induction of 14 heat shock genes (HSGs) (Burg *et al,* 2007). miRNAs may inhibit repressors of Nrf2 by targeting KEAP1, boosting antioxidant enzymes to counter salt-induced ROS. Nrf2 is a transcription factor that activates antioxidant genes, mitigating oxidative damage during dehydration (Wang *et al,* 2016).

Under salt stress conditions, the renal medulla of camels exhibited differential expression of 22 mRNAs, 2 lncRNAs and 31 miRNAs compared to the free salt-intake diet group. The lncRNA *LNC003834 binds to miRNA-34a,* thereby relieving suppression of the salt-absorption-inhibiting *SLC14A1* mRNA from miRNA-34a. This suggests that the lncRNA-miRNA-mRNA act as competing endogenous RNAs (ceRNAs). *SLC6A1, PCBP2* and *PEX5L* were shown to enhance the antioxidant capacity of camel renal medulla cells by reducing reactive oxygen species (ROS) levels. These results indicate that that camels achieve sodium homeostasis through regulating the expression of salt-reabsorption-related genes in the renal medulla, involving ceRNAs (*SLC14A1, LNC003834* and *miRNA-34a*) and antioxidant genes (*SLC6A1, PCBP2* and *PEX5L*) (Zhang *et al,* 2020).

Oxidative stress promotes lipid peroxidation and the formation of pro-inflammatory isolevuglandins (IsoLG). lncRNAs, which play a crucial regulatory role in gene expression, including vascular endothelial cells and immune cells. In hypertension, the decreased transcriptional activity of nuclear factor erythroid 2-related factor 2 (Nrf2 or Nfe2l2) correlates with heightened oxidative stress in antigen-presenting cells (APCs) and impaired control of various antioxidant genes (Khan *et al,* 2024).

Camel-Specific ncRNA Innovations

Camel-specific ncRNAs play integral roles in their legendary resilience by orchestrating gene regulatory networks that manage osmotic stress, conserve water and sustain metabolic function.For example, the camelid-specific miR-2434 exhibits multifunctional regulation: it targets SLC12A3 to suppress renal sodium reabsorption while inhibiting AQP3 expression in kidneys to minimise water loss. miR-2434 is implicated in regulating antioxidant pathways, though its direct link to Nrf2/KEAP1 remains uncharacterised. It may interact with stress-responsive genes in a tissue-specific regulatory effects through interactions with stress-responsive genes (Wang *et al,* 2022).

ncRNAs contribute to camels' drought resilience through multi-level regulatory mechanisms involving metabolic modulation, osmoregulation and stress response pathways. ncRNAs play a pivotal role in the drought adaptation mechanisms of camels through various regulatory processes. These insights

underscore ncRNAs' evolutionary significance in desert adaptation, particularly highlighting, the need for functional characterization of camel-specific ncRNAs and the investigation of their interactive networks with both upstream and downstream proteins.

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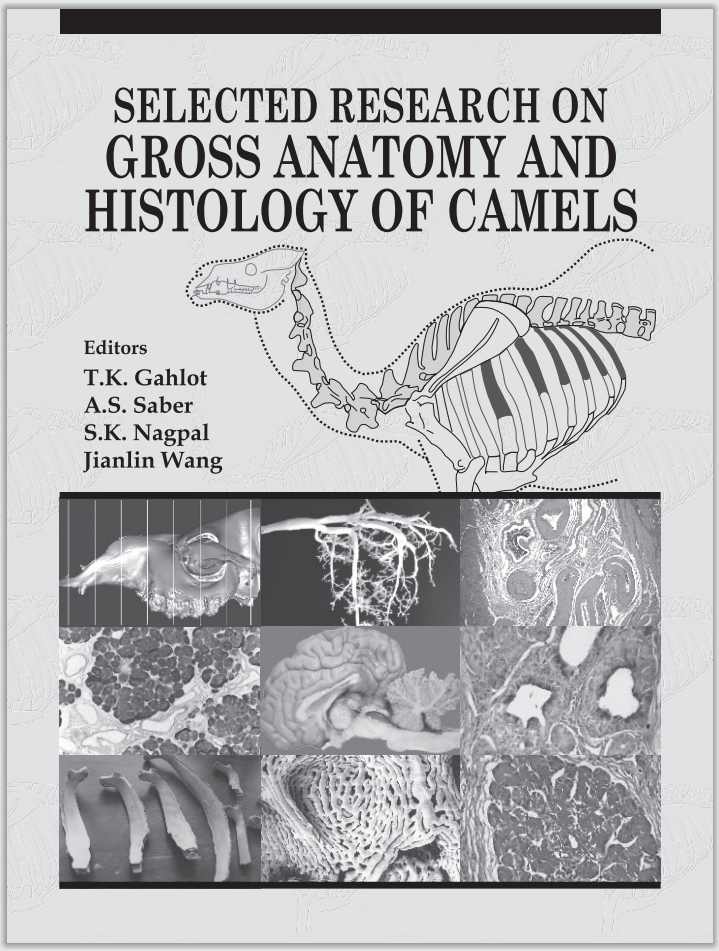
Selected Research on Gross Anatomy and Histology of Camels is a unique reference book on anatomy of dromedary and bactrian camels. This book contains a first ever wide spectrum of histological description of various organs of camels which is depicted by special stains and scanning electronmicroscopy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



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TARGET-CENTRIC APPROACHES FOR CAMEL MILK ADULTERATION DETECTION: A REVIEW OF PROTEIN, METABOLITE AND GENE BASED ANALYTICAL STRATEGIES

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ABSTRACT

Milk fraud is a critical challenge for food safety and the dairy industry. To ensure product authenticity, various detection technologies have been developed and implemented. These methods cover key analytical targets such as proteins, lipids, small molecule metabolites, aromatic compounds and DNA and incorporate detection techniques such as chromatography, spectroscopy, immunology and biosensors. This review article presents an in depth analysis of advances in the field of camel milk authenticity verification studies. This analysis focuses on the evolution of methods for detecting adulteration, taking into account various target components. In addition, the article explores the technological development of detection techniques, highlighting their applicability in a variety of scenarios. The main objective of this study was to provide technical support to promote the sustainable development of the camel milk industry.

Key words: Adulteration detection, biosensors, camel milk, chromatography, ELISA, infrared and raman spectroscopy, isothermal amplification, mass spectrometry, nucleic acid sensing technology, omics techniques, PCR

Dairy products such as milk from cows, goats, sheep and camels serve as vital food sources for humans and various animals. They are rich in essential nutrients including calcium, vitamins and fats (White and Gleason, 2023).

Camel milk is recognised for its significant nutritional and medicinal value. Its components are often compared to human breast milk (Ho *et al,* 2022). In addition, the composition of camel milk, rich in functional proteins and enzymes such as lactoferrin, peptidoglycans, antibodies, lysozyme and lactoperoxidase, gives it remarkable immune and pathogen-resistant properties. Research has shown that casein hydrolysates derived from camel milk have notable inhibitory properties on dipeptidyl peptidase-IV (DPP-IV), suggesting a hypoglycemic action (Su *et al,* 2024). Indeed, the consumption of camel milk is recognised for its benefits to human health, particularly in the prevention of serious diseases (Shakeel *et al,* 2022).

In China, the main camel breeds contribute to the annual national production of camel milk, which amounts to 2,700 tons (China Dataset, 2024). These breeds include the Alax Bactrian camel, the Urad Gobi red camel, the Sunite Bactrian camel, the Xinjiang Tarim Bactrian camel, the Xinjiang Junggar Bactrian camel and the Qinghai Bactrian camel. These breeds have a milk production that can vary between 730 and 1,095 kilograms per lactation period, with an average duration of 14 to 16 months (Xiao *et al,* 2022).

The increase in demand for camel milk has led to a significant increase in the risk of adulteration, jeopardising product quality and consumer safety. Common methods used to prepare these products include adding water or incorporating animal milk of unknown origin, such as cow's milk. While these processes artificially increase the quantity of the products, they significantly reduce their nutritional value. The adulteration of cow's milk

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with contaminants is particularly important because the beta-lactoglobulin present in milk can induce allergic reactions in consumers (Ceniti *et al,* 2023) and pose health risks, including gastrointestinal disorders (Spink *et al,* 2017). These practices not only compromise the authenticity of camel milk but also undermine consumers' right to information. It is therefore imperative to implement an efficient system for detecting adulteration with camel milk.

Milk fraud is a critical challenge for food safety and the dairy industry. To ensure product authenticity, various detection technologies have been developed and implemented. These methods cover key analytical targets such as proteins, lipids, small molecule metabolites, aromatic compounds and DNA and incorporate detection techniques such as chromatography, spectroscopy, immunology and biosensors. This review article presents an in-depth analysis of advances in the field of camel milk authenticity verification studies. This analysis focuses on the evolution of methods for detecting adulteration, taking into account various target components. In addition, the article explores the technological development of detection techniques, highlighting their applicability in a variety of scenarios. The main objective of this study was to provide technical support to promote the sustainable development of the camel milk industry.

Analysis of Adulteration Detection Methods

A. Protein-based method

The protein composition and types of milk from different animal sources exhibit significant differences and these specific proteins can serve as target molecules for species identification. The primary markers for detecting adulteration in dairy products include κ-casein, αs1-casein, α-lactalbumin and β-lactoglobulin. The utilisation of detection technologies founded upon protein targets (e.g., immunochemical analysis, electrophoresis, chromatography and mass spectrometry) has emerged as a pivotal method for the analysis of the species composition of raw milk. This preeminence can be attributed to their exceptional specificity and sensitivity (Jia *et al,* 2025).

1. ELISA

Bovine β-lactoglobulin constitutes 7–12% of total milk protein and 43.6–50.0% of whey protein. Given the absence of this protein in camel milk, it is widely used as a marker for bovine milk adulteration in camel milk. Chi *et al* (2024) developed an enzyme-linked immunosorbent assay (ELISA)-based detection method using bovine β-lactoglobulin (β-Lg), successfully achieving quantitative analysis of trace amounts of cow's milk adulteration in camel milk. This method demonstrates high sensitivity (linear detection range of 1%-100%) and is minimally affected by temperature and pH fluctuations (Chi *et al,* 2024).

Nanoantibodies (Nb), defined as the recombinant variable regions of camelid heavy-chain antibodies, possess an approximate molecular weight of 15 kD, which is approximately one-tenth that of traditional IgG antibodies. This characteristic, coupled with their ultra-small molecular size, enables efficient penetration of biological barriers and binding to hidden epitopes, thereby significantly enhancing detection sensitivity. Rodríguez-Camejo *et al* (2023) developed a highly sensitive sandwich enzyme-linked immunosorbent assay (ELISA) method utilising this characteristic, achieving a quantification limit of 40 picograms per millilitre (pg/mL). This method utilises direct enzyme/fluorescein labeling, thereby obviating the necessity for secondary antibodies and markedly reducing detection time (Rodríguez-Camejo *et al,* 2023). It is imperative to acknowledge that the aforementioned methodologies are contingent upon intact protein conformation epitopes. However, commercially available camel milk products (e.g., powdered milk and liquid milk) undergo high-temperature and high-pressure processing, which has the potential to destroy key epitopes and lead to false-negative results. For instance, Villa *et al* (2022) reported that an indirect enzyme-linked immunosorbent assay (ELISA) method employing rabbit anti-β-lactoglobulin antibodies was wholly incapable of quantifying β-lactoglobulin content in high-pressure sterilised products.

Subsequent studies by Khuda *et al* (2012) have corroborated the notion that processing methodologies exert a substantial influence on the recovery rate and variability of results for casein and β-lactoglobulin in the detected substances. Molecular dynamics simulation technology offers an innovative solution to this problem by virtue of its ability to precisely simulate the effects of temperature and pressure on the conformation of target substances during processing (Zhang *et al,* 2024). Consequently, researchers employed a novel approach that integrated molecular dynamics simulations to screen for stable epitopes of β-lactoglobulin. This strategy was complemented by the utilisation of an indirect/direct competitive ELISA to assess the epitope-binding capacity of antibodies. The established

enzyme-linked immunosorbent assay (ELISA) method exhibited a limit of detection (LOD) and limit of quantification (LOQ) of 0.25 milligrams per kilogram (mg/kg) and 1.07 mg/kg, respectively. This method successfully detected β-lactoglobulin in 23 commercially available processed products. In a similar way, Feng *et al* (2025) screened for a specific epitope of bovine αs1-casein E207 using bioinformatics and developed a quantitative detection method for buffalo milk adulterated with cow's milk. To this end, monoclonal antibodies were prepared using 10 short peptides as antigens and a competitive enzyme-linked immunosorbent assay (ELISA) system was established. This method yielded an IC50 value of 3.8 μg/mL, with detection limits of 1.6 mg/kg and 15 mg/kg in buffalo milk and milk powder, respectively. The recovery rates exhibited a range from 96.2% to 106%, with relative standard deviations ranging from 7.8% to 12.2% (Feng *et al,* 2025). It is important to acknowledge that ELISA-based detection methods have certain advantages, including high specificity and low cost. However, their reliance on intact protein conformation can result in a high rate of false positives and false negatives, which significantly restricts the technology's potential for widespread application.

2. Chromatography and mass spectrometry method

Chromatography/mass spectrometry (GC/MS) is the core analytical method for detecting adulteration in dairy products. The instrument utilises a chromatographic column to achieve precise separation of target compounds. This separation is based on the differences in distribution between the stationary phase and the mobile phase. The efficacy of this technique is attributable to its incorporation of a pre-separation step, a feature that confers upon it a notable advantage over the ELISA method with respect to quantitative accuracy for single substances. This pre-separation step enables the precise quantification of adulteration levels, a crucial aspect of analytical chemistry. For instance, Li *et al* (2024a) developed a method based on ultra-performance liquid chromatography (UPLC). They used bovine β-lactoglobulin (β-Lg) as a marker to qualitatively and quantitatively detect cow's milk adulteration in camel milk. The method demonstrated adequate linearity within the range of 25–1,000 mg/L, exhibiting an RSD of 0.45% for 30% adulterated samples.

The composition of animal milk systems is intricate and their high fat/carbohydrate content can induce substantial matrix effects, thereby interfering with the accuracy of detection results. More critically, the process often causes denaturation of target proteins (e.g., β-lactoglobulin), significantly reducing the accuracy of intact protein detection. To address this, researchers employed an enzymatic hydrolysis strategy to cleave large-molecule proteins into small peptide fragments. They then combined this with mass spectrometry to screen for highly specific and stable characteristic peptides as markers. This combination significantly enhanced detection sensitivity and interference resistance. For instance, Zhang *et al* (2022) employed characteristic peptides derived from αS2-casein, β-casein and κ-casein as markers to quantitatively analyse eight types of animal milk. The limit of quantification (LOQ) for target peptides was determined to be 5–30 μg/L, with recovery rates ranging from 95.2% to 104.5%, as reported by Zhang *et al* (2022). Gu *et al* (2024) identified 22 characteristic peptides from eight species using UHPLC-Q/Exactive-HRMS combined with BLAST alignment, with detection limits of 0.35%–0.49% for liquid milk and 0.68%–0.73% for powdered milk (Gu *et al,* 2024). Furthermore, Hong *et al* (2022) were the first to apply desorption electrospray ionisation mass spectrometry (DESI-MS) to analyse biomarkers in five types of milk sources (cow's milk, goat's milk, camel's milk, soy milk and oat milk). The method was able to achieve 100% cross-validation accuracy and excellent sensitivity for adulteration levels ranging from 0.1% to 5% v/v by identifying specific markers based on mass spectrometry feature differences and combining them with a linear discriminant analysis (LDA) model. This method does not necessitate complex pretreatment, thereby ensuring high classification accuracy while enhancing detection efficiency. It provides a rapid solution for milk adulteration control (Hong *et al,* 2022). Despite the evident advantages of chromatography and mass spectrometry technology in the realm of food adulteration detection, its implementation remains encumbered by substantial limitations. The substantial financial demands associated with the acquisition and maintenance of chromatography and mass spectrometry instruments serve as a considerable economic hindrance. Concurrently, the stringent operational requirements, which demand a high degree of expertise from the operators, act as a significant impediment to the technology's broader dissemination and utilisation. The implementation of this technology in the domain of adulteration detection is significantly constrained by two major restrictions.

3. Biosensors method

In recent years, sensing detection technology has emerged as a key development direction in the field of adulteration detection due to its significant advantages, including label-free operation, simple sample pretreatment, rapid response and high sensitivity. The instrument's detection limit is reported to be at the ng/mL level, with results typically obtained within minutes. Furthermore, the technology's portable and low-power devices are propelling the transition of detection operations from laboratories to on-site rapid quantitative applications. In specific applications, Gao *et al* (2022) developed a sensor based on a porous silicon Bragg reflector. The sensor is capable of detecting β-lactoglobulin by meticulously monitoring alterations in fluorescence and refractive index, which are induced by the reaction of the target biomolecule before and after detection. The device's analytical sensitivity is demonstrated by its capacity to detect β-lactoglobulin in camel milk, with a minimum detection limit of 0.12 nanograms per millilitre. This feature, along with its affordability and high-throughput capabilities, positions the device as a promising tool for analytical chemistry research and applications (Gao *et al,* 2022). In pursuit of higher sensitivity, Li *et al* (2022) constructed a label-free photoelectrochemical immunosensor based on quantum dots (QDs) for detecting β-lactoglobulin. The QDs effectively enhanced the photoelectrical response, enabling the sensor to achieve a detection limit of 0.88 pg·mL^{-1} (Li *et al,* 2022). Meng *et al* (2024) successfully prepared a high-performance molecularly imprinted polymer (MIP) sensor for detecting β-lactoglobulin. The sensor was created using trypsin as a template removal agent. The electrochemical sensor has been demonstrated to detect β-lactoglobulin in a concentration range of 4 to 100 nanograms per millilitre (ng/mL), with a detection limit as low as 3.58 ng/mL (Meng *et al* (2024). It is noteworthy that fluorescence sensing modes generally exhibit superiority over electrochemical detection with regard to sensitivity and accuracy. In order to surmount the constraints imposed by matrix interference in the domain of fluorescence detection and to further capitalise on its advantages, Li *et al* (2024) pioneered a photothermal detection strategy founded upon fluorescence sensing. This strategy culminated in the development of a fluorescence-photothermal dual-mode immunosensor based on nanobodies. This design effectively eliminated matrix interference on the fluorescent signal, with performance characteristics of a detection limit of 0.034 nanograms per millilitre (ng/mL) in fluorescent mode and 0.075 ng/mL in photothermal mode (Li *et al,* 2024). The advantages of this sensing detection technology extend beyond the detection of β-lactoglobulin, as the detection targets can be flexibly switched. For instance, it can be utilised to detect proteins that are unique to cow's milk but absent in camel milk, such as κ-casein. Kourti *et al* (2024) developed an immunosensor chip with a distinctive dual-channel configuration, capable of distinguishing between κ-casein and bovine serum albumin in cow's milk. This innovation enabled the chip to significantly reduce background interference, a significant advancement in the field. This method does not necessitate sample pretreatment and can detect milk adulteration as low as 0.05% by volume within 12 minutes. It exhibits over 10 times the sensitivity of existing ELISA techniques (Kourti *et al,* 2024). In an effort to expedite on-site screening, Sharma *et al* (2021) developed a competitive lateral flow immunoassay. This assay was based on the principle of lateral flow immunoanalysis, utilising non-immunoglobulin antigens and carbon nanoparticles to rapidly detect buffalo milk adulteration in cow's milk. In practice, polyclonal antibodies specific to buffalo milk proteins are conjugated to carbon nanoparticles and coated onto a binding pad; the test line is immobilised with buffalo milk casein at a concentration of 1.6 µg/cm; and the control line is goat anti-rabbit antibody immobilised at 0.5 µg/cm. During the testing procedure, the sample is to be mixed with borate buffer solution and added dropwise. The underlying principle of this method is as follows: in the absence of the target buffalo milk protein in the sample, the test line will appear black or gray; if the target protein is present, the test line signal will weaken or disappear due to competitive binding. This method achieves a sensitivity of 5% adulteration rate, with detection taking only 5 minutes, making it suitable for milk collection stations and heat-treated milk samples. In sum, this method provides a powerful tool for rapid on-site screening (Sharma *et al,* 2021).

B. Based on characteristic metabolites method

Characteristic metabolites refer to compounds other than proteins and nucleic acids found in biological organisms, such as carbohydrates, lipids and other small molecules. Due to the variation in the composition of these metabolites across different animal milk sources, they can function as distinctive indicators for detecting adulteration in dairy products. Among these, spectroscopic analysis is a representative technique for adulteration

detection based on characteristic metabolites. This technology is characterised by its ease of operation and its ability to produce rapid detection results, thereby markedly enhancing detection efficiency. Spectroscopic techniques frequently employed in adulteration detection include infrared spectroscopy, Raman spectroscopy and Omics techniques.

1. Infrared spectroscopy

The foundation of near-infrared (NIR) spectroscopy is the absorption characteristics of substances within the near-infrared region at designated wavelengths. Through the analysis of these absorption spectra, the chemical composition of the substance can be ascertained. This technology utilises the principle of light detection, typically without the necessity of complex sample pretreatment, to discern the absorption, reflection, emission, or scattering response of light by the sample. This enables rapid screening, non-destructive testing and comprehensive characterisation of the sample. For instance, Yuan *et al* (2022) employed a near-infrared spectrometer to obtain spectral data from 43 samples of camel milk mixed with hydrolysed protein and established a detection model based on 33 of these data points. The model demonstrated adequate linearity within the concentration range of 0.01–2 g hydrolysed protein/100 mL camel milk, exhibiting a correlation coefficient R^2 of 0.95 (Yuan *et al,* 2022). Given the inherent complexity of near-infrared spectral data, multivariate statistical analysis of the dataset is typically required and chemometric tools are employed for this purpose (Wang *et al,* 2019). Common methods employed in adulteration detection include principal component analysis (PCA), partial least squares (PLS) analysis and multivariate curve resolution-alternating least squares (MCR-ALS) analysis. For instance, Pereira *et al* (2020) employed a combination of near-infrared spectroscopy (NIR) with partial least squares discriminant analysis (PLS-DA) and the projection search algorithm (iSPA-PLS) to achieve both qualitative and quantitative detection of milk adulteration. This method achieved a detection limit as low as 1.0154 g/100 g (approximately 1%) for milk, with an accuracy rate of up to 100% (Pereira *et al,* 2020). Another study combined near-infrared (NIR) spectroscopy with chemometric methods to successfully perform adulteration quantitative analysis on infant formula containing urea, melamine and starch. The study established a quantitative model based on partial least squares regression (PLS) combined with feature wavelength selection, demonstrating excellent predictive performance with a prediction set coefficient of determination R^2 greater than 0.987 (Zhao *et al,* 2022). However, the primary constraint of PLS is its requirement for a substantial sample size to ensure precise calibration and the necessity to circumvent multicollinearity issues among components.

Support vector machines (SVM), a supervised learning algorithm, can be trained with fewer samples compared to other machine learning classifiers, a quality that lends to its wide applicability. For instance, Buoio *et al* (2024) proposed a hybrid method combining near-infrared spectroscopy (NIR) with variable clustering-support vector machines (VC-SVM) for milk type discrimination, achieving 100% classification specificity.

The advent of artificial intelligence technology has precipitated a paradigm shift in the realm of instrument detection, wherein the integration of multi-source data and the implementation of information fusion technology have emerged as pivotal strategies. The integration of diverse data sources facilitates a more comprehensive and accurate analysis, thereby significantly enhancing the accuracy of adulteration detection. In recent years, significant advancements in artificial intelligence and digital algorithms, particularly when combined with spectroscopic techniques, have revitalised this field. For instance, Colak *et al* (2025) employed a combination of Fourier transform infrared (FTIR) spectroscopy and artificial intelligence (AI) algorithms to detect adulterated milk in buffalo milk. A comparative analysis was conducted of the physicochemical and microbiological characteristics, as well as the spectral data, of 13 concentration mixtures ranging from 0.2% to 10%. In this analysis, six artificial intelligence (AI) algorithms and chemometric methods, including SIMCA and DD-SIMCA, were evaluated. Subsequent to the screening of key spectral features utilising particle swarm optimisation (PSO), the model founded on the ensemble bagged tree algorithm accomplished an accuracy rate of 90.38%. The findings suggest that the integration of FTIR with AI facilitates the effective detection of adulteration, offering a rapid and convenient approach for adulteration detection in the food testing domain (Colak *et al,* 2025).

Although, near-infrared spectroscopy (NIR) has been widely applied in adulteration detection in fields such as food and pharmaceuticals due to its rapid and non-destructive advantages, its spectra primarily reflect the harmonic and sum vibrations of molecules,

resulting in relatively broad and overlapping absorption peaks, which to some extent limit its resolution capabilities. Conversely, mid-infrared spectroscopy (MIRS) is predominantly characterised by the fundamental vibrations of molecules, manifesting as rich and sharp absorption bands with pronounced absorption intensity. This approach has been demonstrated to yield a more substantial and distinctive array of frequency and intensity data. Furthermore, the characteristic vibration peaks of typical functional groups are predominantly concentrated in this region. The unique absorption characteristics of MIRS offer significant advantages in material identification and quantitative analysis. For instance, Feng *et al* (2019) employed mid-infrared spectroscopy to detect milk powder adulterated with rice flour or soybean flour. By establishing a regression model using partial least squares, the researchers were able to simultaneously detect the content of adulterants, with sensitivity and specificity both exceeding 90% (Feng *et al,* 2019). Another study successfully identified the quality characteristics of camel milk compared to other animal milks using mid-infrared spectroscopy. By comparing conventional nutritional components, β-casein and trace element content, the study confirmed the significant uniqueness of camel milk (Li *et al,* 2024b). Chu employed mid-infrared spectroscopy (MIRS) in conjunction with contemporary statistical machine learning methodologies to accurately detect adulteration in cow's milk or water in buffalo milk (BM), goat milk (GM) and camel milk (CM) (adulteration ratios ranging from 5% to 50%). In comparison with conventional methodologies such as partial least squares (PLS), support vector machines (SVM), projection pursuit regression (PPR) and Bayesian regularised neural networks (BRNN), machine learning exhibited superior performance, markedly enhancing the detection accuracy of MIRS for adulteration in high-value dairy products (Chu *et al,* 2023). In addition to upgrading models, studies have centered on integrating mid-infrared spectroscopy with fluorescence spectroscopy, in conjunction with multivariate analysis, to detect three types of animal milk adulteration in camel milk. This approach has enabled the detection of adulteration concentrations as low as 0.5% v/v (Boukria *et al,* 2023). This foundation is being built upon with advancements in computational power and machine learning methods, which are enabling the increased application of multivariate models to enhance the performance of infrared models. For instance, Yao *et al* (2023) integrated conventional statistical methodologies with state-of-the-art machine learning techniques, thereby markedly enhancing the precision and dependability of Fourier Transform Mid-Infrared Spectroscopy (FT-MIR) in the identification of adulterants in camel milk, achieving a minimum detection threshold of 3.27 g of cow's milk per 100 g of camel milk. (Yao *et al,* 2023).

2. Raman spectroscopy

Raman spectroscopy is a molecular vibration spectroscopy technique based on the Raman scattering effect. Its popularity in the field of analysis stems from its high sensitivity, minimal sample requirements, fast analysis speed and ease of operation. A notable advantage of this method over infrared spectroscopy is its low sensitivity to moisture. In the context of analysing liquid milk with elevated water content, infrared spectroscopy frequently obscures signals from other compounds due to the pronounced O-H stretching vibration absorption exhibited by water molecules. Conversely, Raman spectroscopy demonstrates a negligible response to water molecules and minimal interference from water media, rendering it especially well-suited for direct analysis of liquid samples. This advantage has been effectively applied in the domain of liquid milk testing. For instance, Ni *et al* (2023) utilised a combination of Raman spectroscopy with lactose index screening (LIS) and support vector machine (SVM) technology to achieve rapid and accurate identification of pasteurised milk and UHT milk, with a classification accuracy rate of 100% (Ni *et al,* 2023). Furthermore, Raman spectroscopy has exhibited remarkable efficacy in the analysis of powdered samples. Zhang *et al* (2025) employed Raman spectroscopy in conjunction with the Multi-Class Classifier model to effectively differentiate between various types of animal-derived powdered milk, with the model demonstrating an accuracy exceeding 93% for each type of powdered milk. In addressing the more challenging cross-species domain shift issue in milk powder adulteration detection, Ruan *et al* (2024) innovatively introduced meta-learning methods. Their study demonstrated that in a detection task involving 11 adulteration ratios, the identification rate for pure camel milk powder reached 98.92%. It is noteworthy that the meta-learning method attained an overall accuracy of 84.4%, signifying a substantial enhancement of 24.67% in performance when compared to SVM (Ruan *et al,* 2024). In order to enhance detection sensitivity and satisfy the requirements of trace substance analysis, surface-enhanced Raman spectroscopy (SERS)

technology was introduced. This technology utilises the "hot spot" effect generated by metal nanoparticles to significantly amplify the intensity of Raman signals. For instance, Li *et al* (2017) developed a method based on silver nanoparticle (Ag NP) monolayer thin film SERS substrates for the rapid detection of melamine in milk. SERS methods based on gold nanoparticles (AuNPs) have been employed for the determination of urea in milk, with a correlation coefficient R^2 of 0.9873 for the quantitative model (Hussain *et al,* 2019).

3. Omics techniques

Omics technologies provide powerful tools for in-depth analysis of the complex compositional differences between various animal milks and enable more precise detection of adulteration. This technology encompasses multiple levels, revealing the molecular fingerprints of dairy products from various dimensions. At the metabolomics level, the technology focuses on analysing fundamental differences. The analysis of raw camel milk (RCM) and heat-treated camel milk (HCM) was performed using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS). The present study utilised ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS) to analyse camel milk powder (CMP). This analytical approach enabled the identification of 119 significantly different metabolites across key categories, including carbohydrates, glycosaminoglycans, adenosine and phospholipids. This provides significant scientific evidence for the authenticity verification and process impact assessment of camel milk products (Li *et al,* 2022). Building on this, the field of lipidomics seeks to establish species-specific fingerprint profiles: In their seminal study, Wang *et al* (2025) pioneered the application of supercritical fluid chromatography-quadrupole time-of-flight mass spectrometry (SFC-Q-TOF-MS) to the systematic separation and identification of triglyceride (TAG) components in milk derived from six different livestock species. The breeds of livestock present include the Bactrian camel, Holstein cow, goat, Mongolian horse, yak and water buffalo. The triglyceride fingerprinting models constructed through chemometric analysis exhibited a high degree of similarity with species taxonomy and demonstrated excellent accuracy in both internal and external validation. This work presents an innovative lipidomics approach for authenticating the species origin of dairy products (Wang *et al,* 2025). In order to enhance analytical throughput and dimensionality, multi-component simultaneous analysis techniques have been demonstrated to be advantageous. Piras *et al* developed a droplet homogenisation technique based on atmospheric pressure MALDI-Q-TOF mass spectrometry, innovatively achieving simultaneous quantitative analysis of metabolites, lipids and proteins in milk. When employed in conjunction with machine learning software, this method has been shown to achieve 100% accuracy in the precise identification of cow, sheep, goat and camel milk species, thereby significantly enhancing the efficiency of multidimensional analysis (Piras *et al,* 2021). Concurrently, the exploration of novel non-protein detection markers has emerged as a pivotal research direction aimed at enhancing the specificity and sensitivity of detection. This pursuit is driven by the recognition of limitations inherent to traditional protein markers, including thermal stability and species cross-reactivity. In response, researchers are actively pursuing the identification of new detection targets, thereby expanding the repertoire of available analytical methods. In contrast, Li *et al* (2023) adopted a different approach, establishing a detection method for goat milk adulterated with cow milk based on the non-protein marker N-acetylglucosamine. The study revealed that the concentration of this biomarker in milk (146.7 mg/L) is 32 times higher than in goat milk. The method demonstrated good linearity within a 1–100% adulteration range, with a detection limit as low as 0.3%, by optimising pretreatment (cold acetone removal of milk proteins) and detection methods (silylation derivatisation-GC-MS detection, HPLC-MS/MS quantification). The method features simple pretreatment (approximately 10 minutes per sample) and rapid and precise advantages, effectively demonstrating the feasibility and efficiency of non-protein biomarkers for milk adulteration quantification (Li *et al,* 2023). In conclusion, flavouromics offers a distinctive supplementary dimension for the tracking of milk products. Zhou *et al* (2025) combined headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) and headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) technologies to systematically analyse the flavour characteristics of commercially available yak, donkey, camel, goat and cow milk powders in China. The study identified 141 volatile compounds and, by integrating multivariate statistical models such as PCA and OPLS-DA, successfully achieved traceability differentiation among these five types of milk powder, offering a new perspective for dairy

product identification based on flavour fingerprints (Zhou *et al,* 2025).

C. Gene-based methods

In comparison with metabolite-based methods, somatic cells present in animal milk exhibit the presence of nucleic acids (predominantly DNA), thereby offering a distinctive target for the detection of adulteration. It is imperative to note that DNA exhibits remarkable structural stability under the conditions prevalent during dairy processing, including heating and pressure, thereby establishing its reliability as a detection indicator. Gene-based adulteration identification technology is a method of analysing gene fragments from a target species to determine their relative or absolute abundance. This technology is capable of achieving highly sensitive and specific identification of adulteration in animal milk from different species. Representative technologies currently employed for nucleic acid amplification and detection include polymerase chain reaction (PCR), isothermal amplification technology and nucleic acid sensing technology.

1. PCR technology

Since its introduction in 1985, the first-generation PCR technology has become an important detection method by amplifying DNA templates using DNA polymerase and oligonucleotide primers under periodic temperature cycles. Conventional PCR methods principally depend on agarose gel electrophoresis for the detection of amplified products. For instance, Wu *et al* (2022) employed this method to detect adulteration of camel milk with cow's milk and sheep's milk, achieving a minimum detection limit of 1%. While this method boasts advantages such as sensitivity, accuracy and low cost, its operational process is cumbersome, involving multiple steps such as gel preparation and electrophoresis and it cannot monitor the amplification process in real time.

The advent of real-time fluorescent quantitative PCR (RT-qPCR) technology in 1992 provided a solution to this challenge. This technology incorporates fluorescent reporter groups into the PCR reaction system, thereby enabling real-time monitoring of the amplification process. When utilised in conjunction with reference genes or standards, it facilitates a streamlined, one-step quantitative assessment of target gene content or adulterant concentration within the specimen. For instance, Wajahat *et al* employed a qPCR method reliant upon SYBR Green fluorescent dye to detect sheep milk and cow milk adulteration in camel milk, thereby achieving a substantial enhancement in sensitivity with a detection limit of 0.1% (Wajahat *et al,* 2022). Furthermore, the integration of multiplex-specific primers within a singular reaction system enables the efficient simultaneous amplification of multiple target genes by qPCR. The triple real-time fluorescent quantitative PCR method developed by Sarkar and associates is a paradigmatic application. This method is predicated on specific fragments of the cytochrome b gene from cattle, sheep and camels, thereby enabling the simultaneous detection of cow's milk and sheep's milk components in camel milk, with detection limits of 2×10^{-2} ng/μL and 2×10^{-2} ng/μL, respectively (Sarkar *et al,* 2024). In the context of PCR testing, researchers frequently select either mitochondrial genes or nuclear genes as targets. In addition to mitochondrial genes, nuclear genes are also frequently utilised. For instance, Wang *et al* (2020) developed a novel quantitative PCR detection system for camel milk adulteration based on a single-copy nuclear gene. This system effectively addresses the quantitative bias that is often observed in traditional mitochondrial genes due to their multi-copy nature. It accomplishes this by leveraging a single-copy housekeeping gene that functions as both a camel species-specific gene and an internal control gene. A linear model was established between camel milk content and the ratio of Ct values (specific gene Ct/internal control gene Ct), with an R^2 value of 0.9614. The validation of the simulated adulterated sample demonstrated recovery rates ranging from 90% to 120%, exhibiting a coefficient of variation of less than 10%, thereby demonstrating a harmonious convergence of accuracy and precision. The normalisation PCR system, which is based on single-copy nuclear genes, provides a reliable technical foundation for determining the content of camel milk and assessing penalties for adulteration (Wang *et al,* 2020). Nevertheless, conventional quantitative polymerase chain reaction (qPCR) remains constrained in its capacity for absolute gene quantification. To address this issue, digital PCR (dPCR) technology has emerged as a highly sensitive nucleic acid molecular absolute quantification technique. dPCR does not rely on internal reference genes; rather, it achieves absolute quantification by directly counting the fluorescent signals of the target gene. For instance, Li *et al* (2023) employed dPCR technology to detect adulteration in camel milk. This technology subdivides the PCR reaction system into tens of thousands of autonomous micro-reaction

units (e.g., droplets), enabling the calculation of the absolute copy number of the target gene based on the number of units that undergo positive amplification. Despite the fact that dPCR offers extremely high sensitivity, specificity and accuracy and does not rely on Ct values for quantification, it also has some limitations. These limitations include low levels of automation and integration, requiring sophisticated and complex instruments. As a result, dPCR is less suitable for high-throughput testing of large-scale samples.

2. Isothermal amplification technology

Despite the notable benefits offered by thermal cycling amplification techniques, such as polymerase chain reaction (PCR), in terms of nucleic acid precision detection, these methods are often hampered by their reliance on costly thermal cycling instruments, thus constraining their overall applicability. In order to overcome this limitation, researchers have developed isothermal amplification techniques. This technology facilitates the specific amplification of target nucleic acid sequences at a constant temperature, expeditiously producing large numbers of copies to meet detection requirements. Among the array of techniques utilised, loop-mediated isothermal amplification (LAMP) stands out as a prominent and frequently employed method. The reaction is known to occur at a constant temperature of approximately 60°C and is characterised by a brief duration of completion. For instance, Yu *et al* (2021) employed LAMP technology to successfully detect five animal milk-derived genes, including those from camel milk, with a minimum detection limit of 0.05 ng/µL. In simulated adulteration experiments, this method demonstrated the capacity to detect as little as 2.5% (v/v) of cow's milk components in camel milk (Yu *et al,* 2021). However, conventional LAMP detection methodologies frequently necessitate the opening of the reaction tube to introduce dye or undertake endpoint detection, a process that can readily result in aerosol contamination of the amplification products. In order to address this issue, researchers integrated LAMP amplification with lateral flow strip (LFS) detection functionality into a closed, fan-shaped, self-driven microfluidic (SDM) system. This system necessitates only a constant-temperature heating plate (e.g., equipped with a portable power source) and does not require an external power supply to complete detection within one hour. This capability enables contamination-free, visual interpretation of cow's milk adulteration as low as 1% in camel milk.

In addition to LAMP, Recombinase Polymerase Amplification (RPA) is another significant isothermal technology. In contrast to LAMP, which generally necessitates 4–6 primers and functions at elevated temperatures (~60°C), RPA exhibits more accommodating temperature requirements (37–42°C). The core mechanism of this system involves the use of recombinase and polymerase to rapidly amplify target gene sequences at constant temperatures. The shortest reaction time is controllable within 30 minutes. For instance, Zhou and associates have developed a specific RPA method for camel, cow and sheep milk. This approach exhibited excellent specificity, with the capability of detecting as low as 10 pg/µL genomic DNA and high sensitivity when testing milk powder samples. The entire detection process was completed within 30 minutes (Zhou *et al,* 2024). In order to further enhance the accuracy and usability of RPA, researchers are continuously upgrading the technology. A significant advancement in the field is the integration of the CRISPR/Cas12a detection system with RPA technology by Huang and associates this combined method enables direct visualization of milk adulteration results under UV light, thereby significantly simplifying the operational process (Huang *et al,* 2023).

In summary, isothermal amplification technology can be efficiently performed at ambient temperature without reliance on sophisticated temperature control equipment. This approach not only leads to a substantial reduction in testing costs but also genuinely fosters the advancement of nucleic acid testing towards portability and on-site testing.

3. Nucleic acid sensing technology

In order to address the pressing need for simple, low-cost, rapid and on-site nucleic acid testing, nucleic acid sensing technology (also known as gene sensing technology) has emerged. This technology utilises specific nucleic acid sequences (ssDNA probes) as sensitive elements, converting the hybridisation between the probe and target nucleic acids (such as species-specific genes in dairy products) into detectable optical, electrical, or acoustic signals via a transducer. This enables direct or indirect detection of the target.

Fluorescence sensing has become the most widely used nucleic acid sensing method due to its stability. For instance, the daPCR-CHA-FB detection method developed by Thanakiatkrai and associates (which integrates digital PCR-assisted hybridisation chain reaction with fluorescence

signal readout) can detect bovine DNA in dairy products within two hours. This method does not necessitate the purification of DNA or the use of costly equipment and it has a detection limit as low as 0.01 nanograms of DNA (Thanakiatkrai *et al,* 2024). Nevertheless, to achieve true portability and visualisation, paper-based colourimetric sensing demonstrates unique advantages. Researchers developed a paper-based sensing platform based on gold nanoparticle-streptavidin complex colourimetric detection for the visual detection of bovine DNA which was demonstrated to detect adulteration levels as low as 0.01% (v/v) in a binary mixture system of dairy products, with results that are discernible to the naked eye (Bougadi and Kalogianni, 2020). A significant benefit of nucleic acid sensing is its ability to recognise molecules with high sensitivity, allowing for amplification-free detection. Researchers ingeniously utilised the chain displacement and polymerisation functions of the Bst enzyme to promote efficient binding between the probe and target DNA, developing a nucleic acid amplification-free lateral flow strip detection method. This method has been shown to significantly simplify the operational process (by virtue of the fact that it does not require an amplification step), effectively avoid amplification aerosol contamination and improve detection accuracy (Wang *et al,* 2024). In addition to the utilisation of fluorescence and colourimetric methodologies, surface-enhanced Raman spectroscopy (SERS) has been employed for nucleic acid sensing, a process distinguished by its exceptional sensitivity. Researchers employ nanometal particles (e.g., core-shell structured Ag@Au nanoparticles) as SERS substrates, thereby achieving significant enhancement of target DNA signals and effective suppression of background noise from complex matrices (e.g., milk). Researchers combined the trans-cleavage activity of CRISPR/Cas12a (for signal amplification) with SERS detection based on Ag@Au nanoparticles to achieve ultra-sensitive detection of trace DNA in milk, with a detection limit as low as 224 Amol on a portable Raman spectrometer (Pan *et al,* 2022).

Conclusion

Due to its high value, camel milk is currently one of the dairy products most affected by adulteration and mislabeling issues. Various analytical techniques have been developed to detect adulteration in camel milk. The most appropriate technique should be selected based on the source of the adulterants and specific requirements. This paper reviews existing research and categorised camel milk adulteration detection methods into three main strategies: protein-based, metabolite-based and gene-targeted. Widely applied techniques include chromatography, spectroscopy and advanced sensing detection technologies. While these techniques offer high sensitivity and accuracy, significant challenges remain when applied to camel milk adulteration detection. Processing-induced denaturation of target components, degradation of small molecules and interference from complex food matrices can severely impact the reliability of detection results.

Looking ahead, camel milk adulteration detection methods are evolving toward integration and simplification. Advancements in materials science and artificial intelligence algorithms are expected to make existing detection technologies more cost-effective and efficient, providing stronger technical support for ensuring the quality of camel milk.

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THE ROLE OF ACUTE-PHASE PROTEINS AS BIOMARKERS FOR HEALTH AND DISEASE IN CAMELS: A COMPREHENSIVE REVIEW

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ABSTRACT

This review highlights the importance of acute phase proteins (APPs) as key biomarkers in both healthy and sick camels (*Camelus dromedarius*). The study of APPs in camels has gained significant interest in recent years due to their potential roles in health, disease diagnosis and immunological responses. This review provides an overview of their roles in maintaining physiological balance and their possible applications in veterinary diagnostics, examining how APPs are influenced by factors such as age, sex, pregnancy, lactation, postpartum changes and male reproductive aspects. It discusses camels' responses to various clinical conditions, including trypanosomosis, pneumonia, mastitis, urinary and genital tract infections, as well as transportation stress. The APPs act as early indicators of physiological disturbances and disease, making them valuable tools for monitoring animal health. The review also examines the significance of APPs in identifying subclinical infections and monitoring disease progression. Due to their sensitivity to physiological and pathological stimuli, APPs present a promising avenue for advancing camel medicine through early diagnosis, prognosis and treatment monitoring. This review aims to synthesise existing research on APPs in camels, focusing on their biological significance, response to various stressors and potential applications in clinical practice. It also emphasises the need for future research to establish reference ranges, understand species-specific APP responses and develop reliable assays for clinical application. Ultimately, this review highlights the potential of APPs to significantly improve health outcomes and enhance disease management in camels, instilling hope and optimism in their utilisation.

Key words: Acute-phase proteins, biomarkers, camel, diseases, health

The study of acute-phase proteins (APPs) in camels has attracted interest in recent years due to their potential roles in health and, importantly, disease diagnosis. These proteins, acting as biomarkers for various physiological and pathological conditions, are significant in veterinary medicine and animal health management. Various intrinsic and extrinsic stressors trigger acute-phase and inflammatory responses, thereby suppressing the physiological functions of animal tissues (Alotiby, 2024). The increasing awareness of animal welfare and global warming has sparked a growing interest in the relationships between acute-phase protein expression and different stressors, including climate change, malnutrition, injury and infection. Despite camels possessing unique physiological adaptations to maintain homeostasis and to thrive in arid and harsh environments (Ouajd and Kamel, 2009; Fesseha and Desta, 2020; Kandeel *et al,* 2022), all forms of stressors compromise immune function and consequently increase susceptibility to infectious diseases (Al Jassim and Veerasamy, 2015; Allam *et al,* 2017; Hafez and El-Rayes, 2023). Therefore, understanding these relationships is crucial for developing effective strategies to mitigate the impact of stressors on camel health.

The APPs, a group of proteins expressed during the acute phase reaction, hold significant potential as stress biomarkers and serve as a valuable tool for diagnosing inflammatory diseases in animals. Their role in this area has been thoroughly established, providing a strong foundation for their application (Tothova *et al,* 2014; Eckersall, 2019; Alves *et al,* 2020; Bozukluhan and Merhan, 2023). In human medicine, APPs have proven crucial in predicting the effectiveness of cancer therapy and acting as

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reliable markers of successful organ transplantation, highlighting their intertwined potential in cancer treatment and transplantation outcomes (Aoyama *et al,* 2024). Furthermore, Powanda and Moyer (2021) and Tharwat (2023) have proposed that selected APPs should be integrated into future and ongoing clinical studies, opening a promising pathway for understanding disease progression in both acute and chronic conditions in camels and other animal species. This emphasises their potential in veterinary medicine. This optimistic outlook for camel health management, driven by the potential of APPs, should inspire confidence within the veterinary community, encouraging a more proactive approach to health and animal welfare.

At the onset of an inflammatory process, which usually occurs following a physiological condition, acute protein reactant (APR) acts as a non-specific systemic response that rapidly resolves within 1−2 days to restore homeostasis (Eckersall and Bell, 2010; Lakota *et al,* 2011). This complex process involves the upregulation and downregulation of specific positive and negative acute-phase proteins or reactants (APPs or APRs). Haptoglobin (HP), serum amyloid A (SAA), fibrinogen (FB), ferritin (FR), ceruloplasmin (CP), C-reactive protein (CRP) and secretory non-pancreatic phospholipase 2-II (sPLA2-II) are recognised as positive APPs. Conversely, albumin, transferrin, antithrombin (AT), transthyretin (TTR), retinol-binding protein (RBP) and corticosteroid-binding globulin (CBG) are classified as negative APPs.

In hepatocytes, APPs synthesis is a spontaneous reaction to disrupted homeostasis, triggered by the acute phase response (Cray *et al,* 2023). Under normal physiological conditions, acute phase responses stabilise rapidly for a period of several days to weeks. However, the expression of APPs remains altered during stress, transportation, inflammation, infections and chronic diseases in animals (Espinosa *et al,* 2020; Mohamed *et al,* 2021; Razavi *et al,* 2023; Bozukluhan and Merhan, 2023). The classification of APPs primarily depends on changes in their concentration. For instance, a 10- to 100-fold increase is considered major, e.g., SAA and CRP. A 2−10−fold and less than 2−fold increase is classified as moderate and minor APPs α1-acid glycoprotein, FB, HP and CP, respectively (Khalil and Al-Humadi, 2020). Recently, numerous investigators have intensively studied and reviewed APPs variations during various infections and inflammatory states in animals (Alves *et al,* 2020; Bozukluhan and Merhan, 2023; Saco and Bassols, 2023; Smock, 2023; Tharwat, 2023).

The correlation between the strength of APPs, key stress biomarkers and the physiological adaptation of camels to hot environments is a fascinating area of study in camel medicine. Therefore, many researchers have used APPs measurement as a screening test to assess stress, prognosis, diagnosis and treatment of various camel diseases (EL-Bahr and EL-Deeb, 2016; Greunz *et al,* 2018; Tharwat and Al-Sobayil, 2018; Bakhet *et al,* 2021; El-Deeb *et al,* 2022). Therefore, this review aims to update basic knowledge on APPs in camels, suggesting their possible clinical application as a valuable prognostic and diagnostic tool in camel medicine.

Comparative studies indicate that studying APPs in camels can provide insights into the differences and similarities in immune responses across various species, thereby enriching our understanding of animal physiology and immunology (El-Deeb *et al,* 2019; Hussen and Schuberth, 2021). Recent studies on APPs in camels have focused on identifying specific proteins, understanding their roles in the immune response and evaluating their potential as diagnostic biomarkers (Tharwat, 2020; Bakhet *et al,* 2021; El-Deeb *et al,* 2022; Tharwat, 2023). These studies utilised various methods, including proteomic analysis, Enzyme-Linked Immunosorbent Assay (ELISA) and gene expression studies. Proteomic analysis is commonly employed to identify and quantify APPs in camel serum, whereas ELISA measures the concentration of specific APPs. Gene expression studies were conducted to comprehend the regulation of APPs at the molecular level.

Positive APPs

Positive APPs are primarily produced by the hepatocytes and released into the blood in response to cytokine stimulation (Alves *et al,* 2020). Pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumour necrosis factor-alpha and interleukin-1-beta, activate specific receptors on target cells, promoting metabolic changes that lead to local and systemic effects, including APPs (Yoshioka *et al,* 2002; Peterson *et al,* 2004; Tizard, 2018). For instance, IL-6 plays a critical role in the APR by stimulating the hepatocytes to generate positive APPs during inflammation, infection, or other stressors (Ceron *et al,* 2010; Gulhar *et al,* 2023). The APPs are not only essential for the innate immune response in camels but also in other animal species. They also serve as crucial biomarkers for various health conditions, animal welfare and food safety, making them invaluable tools for veterinarians

and researchers (Greunz *et al,* 2018; Tharwat, 2023). Several critical positive APPs, including HP, SAA, FB, FR, CP, CRP and spla2-II, have been identified in camels, with each playing a vital role in health, the body's immune response and diseases (Baghshani *et al,* 2010; El-Deeb *et al,* 2022).

Haptoglobin (HP), an α2-globulin, is a major APP in all animal species (Faye and Bengoumi, 2018; Tothova *et al,* 2014; Crays *et al,* 2023). It significantly enhances the stability of haemoglobin's peroxidase activity (Peterson *et al,* 2004). The HP's protective role against oxidative cascade reactions is associated with cell-free haemoglobin (Hb) and the propagation of oxidative reactions through pseudo-peroxidase activities of Hb, facilitated by binding to free Hb (Alayash *et al,* 2013). The Hb-HP complex, released from erythrocytes, prevents oxidative damage and preserves iron, as well-documented by Banerjee *et al* (2012). This complex, once formed, can be efficiently discharged by receptor-mediated endocytosis through the reticuloendothelial system, a process that reinforces the body's efficient defense mechanisms (Vlasova, 2018).

The primary role of HP, its bacteriostatic effect, is related to the Hb-HP complex properties, as it restricts the free iron accessible to bacteria. Peterson *et al* (2004) have comprehensively reviewed the functions of HP, including its immunomodulatory effects, stimulation of angiogenesis, regulation of fatty acid metabolism, induction of lipid synthesis in the liver and inhibition of neutrophil respiratory burst activity. As a versatile scavenger protein, HP is a fascinating area of study due to its diverse properties, such as antimicrobial, anti-inflammatory and antioxidant effects (Wan *et al,* 2021). The binding of HP to neutrophil integrins CD11b and CD18 produces anti-inflammatory effects. At the same time, its role as an antimicrobial and antioxidant agent is linked to its ability to remove free Hb from the blood, thereby reducing iron availability to pathogens. Notably, as a positive APP, HP levels increase during inflammation. This feature highlights its diagnostic value during intravascular haemolysis and inflammation (Gulhar *et al,* 2023). Elevated HP levels are associated with inflammation, infection and haemolytic anaemia (Cray *et al,* 2009).

According to Nazifi *et al* (2012), the reference values for serum HP in healthy dromedary camels aged between one and five years were 0.11–0.61 g/L, which were higher than those of cattle (0.022–0.047 g/L). Age and sex did not significantly affect serum HP concentration in dromedaries (Nazifi *et al,* 2006; Baghshani *et al,* 2010). Camels showed markedly high HP levels on day 21 postpartum, which contributed to a decrease in both the frequency and severity of illnesses (El-Sayed, 2025).

Numerous studies have highlighted the importance of HP as a clinically valuable parameter for evaluating the occurrence and severity of inflammatory diseases in camels. The HP levels were significantly higher in pneumonic camel calves than in healthy ones (El-Deeb, 2015). Bakhet *et al* (2021) reported substantial HP elevations in interstitial and fibrinous bronchopneumonia compared to control camels, suggesting that HP serves as a diagnostic parameter for pneumonia in camels. In response to parturition stress in dromedary camels, HP values demonstrated a significant increase relative to the values measured pre- and post-partum, indicating a normal physiological response to the release of cortisol-stress hormone (Tharwat and Al Sobayil, 2015). A notable rise in serum HP was also observed in racing camels, highlighting that non-inflammatory and psycho-physical stressors trigger the combined activation of both sympathoadrenal and the hypothalamic–pituitary–adrenal axes (Tharwat and Al-Sobayil 2018; Mohamed *et al,* 2021; El-Deeb and Abdelghani, 2022), suggesting that HP is not merely a biomarker, but a promising tool for assessing the health status of racing camels.

The HP is also considered a vital prognostic and diagnostic biomarker in camels suffering from various infections. Notable increases in HP levels have been documented in camels naturally infected with *Coxiella burnetii*, *Mycoplasma hemolamae*, *Toxoplasma gondii* and *Trypanosoma evansi* (Azma *et al,* 2015; El-Deeb and Elmoslemany, 2015; El-Bahr and El-Deeb, 2016; El-Deeb *et al,* 2019; Al Matwari *et al,* 2022). Camels with paratuberculosis showed significantly higher HP values compared to healthy controls (El-Deeb *et al,* 2014). Other researchers have suggested that HP may serve as a diagnostic and prognostic marker for respiratory and urinary tract infections, Q-fever and abortion in camels (El-Deeb, 2015; El-Deeb and Buczinski, 2015; Allam *et al,* 2017; El-Deeb *et al,* 2019; Bakhet *et al,* 2021; Hafez and El-Rayes, 2023).

Greunz *et al* (2018) showed that camels with chronic *Corynebacterium* lymph abscess had higher HP levels than healthy ones. The authors studied APPs in surgically castrated males treated with a gonadotropin-releasing hormone vaccine for one week. The HP showed a significant increase after vaccination and castration and remained elevated for seven days, with mild variations in the control group.

A study conducted by El-Deeb and Buczinski (2015) involving 74 camels with urinary tract infections revealed significantly elevated levels of HP in sick animals (2.45 g/L) compared to healthy ones (0.31 g/L). Furthermore, an investigation indicated a notable rise in HP values in pneumonic camel calves compared to their healthy counterparts (El-Deeb, 2015).

Fibrinogen (FB) is a precursor to fibrin, which is essential for blood clot formation, helps wound healing and provides a framework for tissue repair (Bayer, 2022). Elevated FB levels indicate inflammation and can be utilised to diagnose inflammatory diseases and conditions associated with tissue damage (Eckersall, 2000). The plasma FB concentration in camels is similar to that in humans, with values ranging from 150 to 400 mg/dL (Abdel Gader *et al,* 2013). Other researchers have provided a reference range for serum FB in apparently healthy dromedary camels of 3.10−3.27 g/l (Baghshani *et al,* 2010; El-Deeb and Buczinski, 2015). The same authors observed that road transport and urinary tract infections significantly raised FB levels in camels.

Numerous studies have demonstrated markedly higher levels of FB in camels suffering from interstitial and fibrinous broncho-pneumonia compared to healthy animals. These findings suggest that FB may serve as a diagnostic indicator of pneumonia in camels (Bakhet *et al,* 2021; Ahmed *et al,* 2021; Hafez and El-Rayes, 2023). In contrast, the concentration of FB was significantly higher in pneumonic camel calves (5.1±1.30 g/L) than in healthy ones (3.3 ± 0.30 g/L) (El-Deeb, 2015). The authors examined the clinical significance of FB as a diagnostic marker or therapeutic target for treating respiratory diseases in camels. Other researchers investigated the effect of walking and road transport on APP production in camels. They concluded that stress from road transport led to increased APP production, as shown by a significant rise in HP and FB (Mohamed *et al,* 2021). Significantly higher FB levels were observed in camels infected with *Mycoplasma hemolamae* than in healthy animals. Additionally, other researchers found that FB levels increased notably between days three and five, with higher levels observed post-castration in camels (Greunz *et al,* 2018). In female camels, El-Sayed (2025) noted significant increases in FB levels at parturition, whereas levels decreased significantly by the 21st postpartum.

Ferritin (FR), a cytosolic protein found in most tissues, is crucial in iron homeostasis. Its primary function is as an iron-carrier protein that regulates the body's iron levels. Small amounts of FR are secreted into the serum, making it an indirect biomarker of the total iron stored in the body. This property makes plasma FR a valuable diagnostic tool for iron-deficiency anaemia (Wang *et al,* 2010).

Ferritin is also a well-known acute-phase reactant, with levels that reflect both acute and chronic inflammation, as well as infectious diseases (Mahroum *et al,* 2021). Importantly, FR plays a key role during inflammation by sequestering iron, limiting its availability to pathogens and providing a protective function (Moreira *et al,* 2020). Furthermore, Gehrer *et al* (2023) highlighted the physiological role of FR in intracellular Fe dynamics. The review provided insights into the novel iron-dependent mechanisms that influence cell growth, differentiation and viability, as well as prognostic and diagnostic biomarkers for infectious and inflammatory disorders. Additionally, it explored how inflammatory mediators and cytokines affect the regulation and expression of FR. Recently, FR has been established as a critical biomarker for diagnosing viral and bacterial infections, inflammation and other infectious diseases (Chen *et al,* 2023).

Kotla *et al* (2022) provided a comprehensive review of the role of FR, emphasising its importance as a key storage protein in systemic, cellular and intracellular iron homeostasis. At the intracellular level, the FR plays a vital role in regulating iron homeostasis by sequestering excess iron and keeping it in a redox-inactive form, thereby making Fe available either during periods of deficiency or increased demand. In clinical medicine, both cellular and systemic levels of FR are critical indicators of iron status and essential biomarkers for inflammatory, immunological disorders and cancer.

Limited data are available on FR concentration in camels. Recently, Aldujaily *et al* (2025) showed that serum FR levels in healthy dromedary camels aged from one to seven years were 349.00±3.98 µg/L. The same authors noted that age did not significantly influence FR concentration. Ghali *et al* (2020) reported that serum FR concentration was significantly higher in adult male Iraqi camels (371.53±5.34 µg/L) compared to females (328.16±14.58 µg/L). In studies of Iraqi camels, FR values were significantly lower in animals with trypanosomosis and mastitis (Al-Rubaie *et al,* 2020; Darwish, 2023). The authors stutied that measuring serum FR could serve as a reliable marker for mastitis and *Trypanosoma evansi* infection. Furthermore, Aldujaily *et al* (2025) showed a strong link between iron deficiency and pica development in

camels, highlighting the correlation to low serum iron concentration, ferritin levels and persistent dietary iron insufficiency.

Ceruloplasmin (CP), a copper-binding protein with oxidase activity, commonly known as serum ferroxidase, accounts for 95% of blood copper transport; therefore, it contributes to iron metabolism and exhibits antioxidant properties (Fleming and Gitlin, 1990; Adamczyk-Sowa *et al,* 2016; Lopez *et al,* 2023). In the hepatocytes, CP is synthesised as a single polypeptide chain and secreted into the blood, where it binds 6 copper atoms per molecule. The CP participates in various biological processes, including copper transport, iron metabolism, tissue angiogenesis and antioxidant activity (Khalil and Al-Humadi, 2020). In protein electrophoresis, CP appears in the γ-globulins fraction, with 95% of plasma containing a copper-bound complex (Neşelioğlu *et al,* 2022). Essamadi *et al* (2002) isolated and purified CP from a 6-month-old young camel. They showed that the molecular weight of CP was approximately 130.000 Da using non-reducing SDS-electrophoresis, with slightly higher electrophoretic mobility than the human protein, indicating that the protein is more acidic and compact, with no differences between the adult and young camels.

Higher CP levels indicated inflammatory responses, infection and liver diseases (Lopez *et al,* 2023). Human studies have demonstrated that changes in copper homeostasis and CP are linked to Alzheimer's disease (Squitti *et al,* 2007). Additionally, CP has been recognised as a useful serum biomarker for chronic demyelinating and neurodegenerative processes, involved in oxidative stress mechanisms rather than an APP (Adamczyk-Sowa *et al,* 2016).

Faye *et al* (1995) previously reported a mean value of 41.4 ± 2.6 UO for CP in female camels over one year old. The authors studied that season, mineral supplementation and health status significantly influenced serum CP concentration. Nazifi *et al* (2000) found that age and sex did not significantly affect CP levels. El-Bahr and El-Deeb (2016), Allam *et al* (2017) and Hafez and El-Rayes (2023) found that camels with *Trypanosoma evansi* infection and respiratory diseases had significantly higher CP levels than healthy camels. During postpartum and in she-camels with endometritis, the CP values consistently exceeded those of healthy females (El-Deeb *et al,* 2022; El-Sayed, 2025).

Serum amyloid A (SAA) consists of a group of APPs predominantly produced in the liver. However, these may also be found in other body systems, including the stomach and intestines, during chronic infection (Den Hartigh *et al,* 2023). Typically, plasma levels of SAA remain low; however, these can increase sharply to 1,000 times their normal level in response to infection, inflammation and trauma (Zhou *et al,* 2016). Consequently, SAA serves as a valuable diagnostic and prognostic marker, as well as a therapeutic biomarker for monitoring infections, trauma, inflammatory responses and cancer (De Buck *et al,* 2016). The SAA attracts immune cells to the site of inflammation and promotes the activity of enzymes that break down the extracellular matrix; therefore, it is considered a highly sensitive marker of inflammation, with levels rising significantly during acute inflammatory responses. As a result, it is believed that SAA play various roles during the acute phase of these conditions. Recent research has also indicated that SAA is involved in immune regulation, particularly T-cell immunity (Chen *et al,* 2023).

Tharwat and Al-Sobayil (2018) investigated the impact of electroejaculation (EEJ) on the concentrations of SAA in camels. The authors found that SAA levels significantly increased in response to EEJ stress. This response activates the combined actions of the hypothalamic-pituitary axis, the sympathoadrenal system and the release of glucocorticoids, along with components of cellular immunity, ultimately enhancing hepatic synthesis and the release of APPs. The elevated levels of SAA, along with corresponding increases in cortisol, are likely a result of physical stress. In camels, Greunz *et al* (2018) indicated that SAA exhibited no change in surgically castrated males treated with a gonadotropin-releasing hormone vaccine for one week.

In female dromedary camels, significant increases in SAA were observed at parturition, compared to values pre- and post-partum, indicating the camel's immune response to parturition stress (Tharwat and Al Sobayil, 2015; El-Sayed, 2025). Significantly elevated levels of SAA have been observed in camels suffering from pneumonia and other respiratory diseases (Allam *et al,* 2017; Hafez and El-Rayes, 2023). The SAA levels were noticeably higher in dromedary camels with lameness due to punctured foot and traumatic injuries than in healthy camels. This suggests that SAA can be an effective diagnostic parameter for monitoring the response to lameness treatment (El-Deeb and Abdelghani, 2022). Chronic infections caused by *Toxoplasma gondii* and *Trypanosoma evansi* in camels have been associated with markedly increased SAA concentrations (Azma

et al, 2015; El-Bahr and El-Deeb, 2016). Additionally, Allam *et al* (2017) found that camels suffering from respiratory diseases had significantly elevated SAA levels compared to healthy camels. Moreover, a study on APPs in pneumonic camel calves revealed significantly higher SAA values relative to healthy counterparts (El-Deeb, 2015; Hafez and El-Rayes, 2023).

C-reactive protein (CRP) is an APP produced by the liver, with levels increasing in response to inflammation, mainly triggered by interleukin-6 (IL-6). It plays a key role in identifying and clearing foreign pathogens and damaged cells by activating the classical complement pathway and aiding phagocytic cells. While it has pro-inflammatory and anti-inflammatory effects, elevated CRP levels can indicate bacterial infections and systemic inflammation (Singh *et al,* 2025). Mouliou (2023) comprehensively reviewed the CRP role in clinical medicine as a recognised biomarker for identifying or excluding inflammation. The author noted that numerous scientific efforts have been aimed at uncovering its direct pleiotropic functions, such as its role as a vital immunochemical marker for various medical issues, including infections like sepsis, diseases, autoimmune disorders, malignancies and other health conditions, which have gained significant popularity.

In humans, CRP has been identified as a key marker of the presence, severity and treatment efficacy of infection (Powanda and Moyer, 2021). The authors noted that a high-sensitivity CRP test was more effective than the standard test and can be used to assess the risk of developing coronary artery diseases. In another study, high-sensitivity CRP has been established as an inflammatory biomarker associated with cardiovascular disease in patients (Osawa *et al,* 2024).

Few studies were conducted in camels to assess CRP as a negative APP. Bakhet *et al* (2021) reported highly significant elevations in CRP in interstitial pneumonia and fibrinous bronchopneumonia compared to healthy camels. The study results concluded that CRP is a diagnostic parameter for pneumonia in camels. Other studies have noted that camels with respiratory diseases have substantially increased serum CRP levels (Hafez and El-Rayes, 2023).

Negative APPs

Negative APPs are those whose plasma concentrations decrease in response to inflammation, infection, or other stressors. In camels, several proteins have been identified as negative APPs: albumin, transferrin, AT, TTR, RBP and CBG.

Albumin, the main negative APP across all mammalian species, is the most abundant plasma protein, constituting approximately 50−60% of total protein. Albumin plays a vital role in maintaining animal health, serving as a nutrient source and regulating osmotic pressure. Hypoalbuminemia, a condition characterised by low albumin levels, can result from protein loss due to kidney or gastrointestinal diseases and/or oedema caused by decreased synthesis linked to liver disease or malnutrition (Gounden *et al,* 2023).

In camels, albumin comprises 35−40 mg/ml of plasma proteins, with essential functions such as regulating blood osmotic pressure, maintaining blood pH and transporting fatty acids, hormones and drugs (Eckersall, 2008; Faye and Bengoumi, 2018). As dromedary camels are well-adapted to hot, dry climates, their plasma osmolality increases during water deprivation, making serum albumin crucial for blood pressure regulation (Malik *et al,* 2013). Furthermore, research examined serum protein and albumin patterns in growing, pregnant camels and during different lactation stages and found that albumin levels varied throughout the growth phase and lactation period (Elkhair, 2024; Adam and Elkhair, 2023). These fluctuations suggest that physiological states, such as growth and lactation and water deprivation can influence albumin concentrations, which may have implications for interpreting albumin levels during health and disease states.

Albumin is recognised as a negative APP in camels, suggesting that its serum levels decline during inflammatory responses. Hypoalbuminemia is part of the APR, during which hepatocytes concentrate on generating positive APPs (HP, SAA and FB) to combat infection or inflammation, leading to a reduction in albumin synthesis (Eckersall and Bell, 2010). Additionally, hypoalbuminemia may occur during inflammation when the liver prioritises the production of positive APPs (Gounden *et al,* 2023). Studying the biochemical aspects of camel albumin, identified as a positive APP, could enhance veterinarians' understanding of camel health, nutrition and disease management (Tharwat, 2023). Consequently, monitoring albumin levels in camels can be a useful diagnostic and prognostic parameter for evaluating the presence and severity of inflammatory conditions, aiding veterinarians in

assessing disease progression and the effectiveness of treatment strategies.

A study by El-Deeb and Elmoslemany (2015) found that urinary tract infections in camels exhibited significantly lower serum albumin levels compared to healthy controls. The authors explained that hypoalbuminemia was accompanied by elevated levels of positive APPs like HP, SAA and FB, indicating an active inflammatory response. Similarly, research on respiratory diseases in camels reported decreased serum albumin levels in affected animals (Greunz *et al,* 2018; Bakhet *et al,* 2021). The studies highlighted that, alongside the reduction in albumin, there were significant increases in pro-inflammatory cytokines and positive APPs, underscoring albumin's role as a negative APP during inflammation. Furthermore, Hafez and El-Rayes (2023) identified the most significant bacteria responsible for pneumonia in diseased camels, which exhibited notable increases in albumin levels, accompanied by substantial increases in APPs, including FB, CP, HP and SAA, compared to the control group.

Transferrin, an iron-binding protein, is an essential component of the body's iron transport system. Its levels decrease during inflammation, reducing iron availability to pathogens. With its strong affinity for f_e 3+ iron, it effectively binds almost all plasma iron, ensuring minimal free iron levels in the body. This process facilitates the delivery of f_e 3+ ions, a crucial function. The transferrin-iron complex, with its impressive turnover rate of approximately ten times per day, efficiently meets the demands of erythropoiesis. Transferrin's role in regulating iron release from the reticuloendothelial system and its absorption by the bone marrow is vital. The systemic iron homeostasis is heavily dependent on the regulation of its absorption, primarily in the proximal portion of the small intestine (Ogun and Adeyinka, 2022). This regulation is primarily facilitated by the binding of iron to transferrin, which transports it to various tissues and organs.

Studies on the role of transferrin in healthy and diseased camels showed that anaemic camels had markedly lower serum iron levels and reduced transferrin saturation percentage compared to healthy ones (Al-Dhalimy and Al-Hadithy, 2017). A study identified iron deficiency in camels, particularly among young males and those with low red blood cell counts and haemoglobin levels, which were associated with reduced transferrin levels (Al-Dhalimy *et al,* 2020). Additionally, camels infected with *Trypanosoma evansi* and those with mastitis demonstrated a significant decrease in total serum iron and ferritin levels and transferrin saturation (Al-Rubaie *et al,* 2020; Darwish, 2023). In contrast, the same authors found that infected male and female camels of different ages exhibited an increase in total iron-binding capacity and unsaturated iron-binding capacity, indicating profound systemic effects on iron metabolism. These findings underscore the diagnostic relevance of transferrin in monitoring camel health and managing disease-related anaemia, inspiring further research and exploration in the field of camel health for veterinarians, animal health researchers and veterinary science students.

Antithrombin (AT) is a serum protein produced by the liver, typically found at concentrations of 0.125 to 0.160 mg/ml. The anticoagulant properties of AT explain the role of heparin in treating and preventing thrombosis, as heparin binds to AT and induces a conformational change that significantly enhances AT's ability to inhibit reactions of the coagulation cascade. Beyond its role in blood coagulation, it also promotes the release of various compounds that help reduce inflammation (Rezaie and Giri, 2020). Regarding coagulation, AT is essential in preventing blood clotting by neutralising thrombin and other protease enzymes involved in the blood coagulation pathway. During acute inflammation, reduced AT levels may lower the risk of excessive clotting during infections. Therefore, AT is a pivotal regulator of coagulation and inflammation, with levels rising during acute inflammatory responses. In camels suffering from pneumonia, notable increases in APPs, such as HP and FR, have been observed, suggesting a corresponding rise in AT during such responses (Greunz *et al,* 2018; Ahmed *et al,* 2021).

In human medicine, optimising AT is a key treatment consideration, especially in high-risk scenarios such as pregnancy, postpartum period, surgery and trauma for individuals with AT deficiency (Rodgers and Mahajerin, 2023). Furthermore, the relationship between inflammation and haemostasis is vital; AT's role in maintaining coagulation balance is crucial, particularly in scenarios like disseminated intravascular coagulation, which is well-documented (Schlömmer *et al,* 2021).

Although, AT is a vital component of the APR, it is essential to emphasise that its levels undergo significant fluctuations in all inflammatory conditions. Notably, there is a lack of studies examining AT as a key APP in camels. Therefore, it is necessary to conduct further research to develop a comprehensive knowledge of its behaviour across different disease

states. Consequently, assessing AT alongside other camel APPs can provide valuable insights into the severity of inflammatory diseases and their impact on coagulation status, potentially transforming our understanding of these conditions.

Transthyretin (TTR), also known as prealbumin, is primarily produced in the liver. It serves as the primary transport protein for thyroxine, retinol-binding protein and vitamin A, showing varying affinities across mammalian species (Tóthová and Nagy, 2018). The TTR is crucial for regulating thyroid hormones, which are responsible for maintaining metabolic balance and thermoregulation in arid regions (Sabatino and Vassalle, 2025).

In humans, measuring TTR concentrations serves as a diagnostic tool for certain diseases; however, it is more commonly used as a nutritional biomarker to evaluate protein-calorie malnutrition, as well as a prognostic indicator in critically ill patients (Dellière *et al,* 2021). A study by Cotrina *et al* (2021) confirmed TTR's role in Alzheimer's disease as a neuroprotective agent through a drug discovery programme focusing on chaperone-like small-molecule compounds that enhance TTR/Amyloid-beta interactions. Furthermore, Ranasinghe *et al* (2022) thoroughly reviewed the function of TTR as a biomarker for disease prognosis, nutritional and refeeding status and protein-energy malnutrition. The same authors noted that TTR is also the only protein significantly linked to changes in lean body mass. Notably, recent research has highlighted TTR's roles in cell biology, particularly in supporting the health of neuronal, central and peripheral nervous systems, influencing and regulating cellular proliferation and fate, metabolism, angiogenesis and cancer (Magalhães *et al,* 2021). The authors explained that TTR's molecular mechanisms may go beyond its carrier functions, including receptor interactions and activation of intracellular signaling pathways.

In veterinary medicine, various studies have assessed TTR values in specific diseases such as protein-energy malnutrition in rats and neonatal calf diarrhoea (Henze *et al,* 2008; Tóthová and Nagy, 2018); however, its importance in animal health is rarely studied. An increase in TTR levels was observed in neonatal calves following colostrum intake; however, it gradually declines until the 3rd month of life, indicating adequate nutrition and liver synthesis (Tóthová *et al,* 2015). In contrast, TTR decreased in dogs with hypothyroidism and chronic renal failure (Piechotta *et al,* 2012; Raila *et al,* 2007). Therefore, further research is required to understand how various diseases affect TTR levels in animals.

Although direct studies on TTR in camels are limited, existing proteomic and serum protein electrophoresis research highlight its vital role in helping these animals physiologically adapt to harsh desert conditions. Furthermore, investigations into the functions of hormone-binding and regulatory proteins suggest that TTR may play a significant part in camel homeostasis by mediating thyroid hormone transport and stabilisation in a thermally stressful environment (Palha, 2002). Studies on serum protein electrophoretic profile of healthy dromedary camels fractionated serum proteins to albumin, α1 and 2, β1 and 2 and γ-globulins; however, TTR was not directly identified, it typically migrates in the prealbumin or alpha-globulin region, indicating its likely inclusion in the profile (Ahmadi-hamedani *et al,* 2014; Elkhair and Hartmann, 2014; Abdoslam *et al,* 2018; Adam and Elkhair, 2023). Furthermore, a comprehensive proteomic analysis of various camel organs demonstrated increased expression of proteins associated with metabolic regulation and cellular stress responses (Warda *et al,* 2013). Therefore, these studies reinforce the concept that camel proteins, including those involved in thyroid hormone transport, are uniquely adapted for stability and function under physiological stress; however, direct genetic characterisation of TTR in camels remains an area for future research, particularly in the molecular and functional expression of TTR in camels, which is warranted to fully elucidate its significance.

Retinol-binding protein (RBP) is the principal carrier protein of vitamin A in the bloodstream, responsible for transporting it from hepatic storage to peripheral tissues. In mammals, RBP usually circulates in a complex with TTR, stabilising the RBP-retinol complex and preventing renal loss, a phenomenon that has been well studied in both humans and various animal species (Steinhoff *et al,* 2022).

Steinhoff *et al* (2022) thoroughly examined the role of RBP in health and disease across human and mouse models, covering aspects such as thermoregulation, behavioural, neurological and cardiovascular functions, adipose tissue lipolysis, liver fat, embryonic development, retinoid homeostasis, insulin sensitivity, glucose tolerance and vision. The authors described how impaired retinal function impacts visual sharpness following birth. This condition typically stabilises within 4 to

5 months with adequate vitamin A intake; however, it does not normalise on a vitamin A-deficient diet. Nonetheless, the role of RBP in camels remains insufficiently studied. Due to its small molecular size, RBP is generally expected to migrate within the alpha-globulin region in electrophoretic patterns. However, the specific detection and quantification of RBP were not performed, emphasising the need for targeted analytical approaches, such as ELISA or mass spectrometry, to characterise RBP in camels.

Although, direct studies on RBP in camels are limited, the existing literature suggests a probable role for RBP in maintaining vitamin A balance, especially under diverse environmental and physiological conditions. Given the camel's unique adaptations to arid environments and the vital roles of vitamin A in immunity and reproduction, future research should focus on characterising RBP at a molecular level and investigating its physiological regulation. Understanding RBP's function in camels could offer more profound insights into their nutritional needs and the mechanisms that support their resilience. A notable study by Ghardan Mashhadi *et al* (2013) demonstrated that healthy camels had higher serum vitamin A levels, likely due to increased availability of green fodder, with remarkably stable levels across seasons. This stability in vitamin A levels, despite seasonal dietary changes, is a testament to the camel's remarkable resilience and adaptability. It also opens up an intriguing avenue for further research, particularly on the functions of RBP in camels, which may attract the interest of many researchers.

Furthermore, Abdelnour *et al* (2019) comprehensively reviewed the role of retinoic acid (RA), a physiologically active metabolite of vitamin A, as a key regulator of cell differentiation, development and *in vitro* embryonic growth in mammals. The review emphasised RA's role in enhancing oocyte nucleus maturation, cleavage and blastocyst formation, attributed to its anti-apoptotic and antioxidant properties against reactive oxygen species, which were achieved by modulating gene expression pathways. The review also suggested that adding up to 50 nM RA can significantly improve mammalian oocyte maturation media, offering a promising potential for the use of RA in assisted reproductive technology, inspiring further research and development in this field.

In camels, Saadeldin *et al* (2019) investigated All-trans RA and its effects on biological processes such as cell growth and fertility in dromedary camels, focusing on its influence on the *in vitro* maturation (IVM) of the cumulus-oocyte complex. Their results indicated that 20 μM RA supported polar body extrusion and oocyte meiosis, decreased the number of degenerated oocytes, reduced mRNA levels of apoptosis-related genes and transcripts associated with cytoskeleton pathways and increased Transforming Growth Factor beta (TGFβ) expression in cumulus cells compared to controls. The authors emphasised the role of RA in enhancing camel oocyte IVM, establishing a basis for further investigation in cumulus expansion mechanisms and meiotic pathways, in particular those linked to TGFβ in cell growth, differentiation and apoptosis. Although, the study did not directly investigate RBP, it underscores the crucial importance of retinol delivery mechanisms, which likely involve RBP, in reproductive function.

Corticosteroid-binding globulin (CBG), a high-affinity glycoprotein primarily synthesised by the liver and secreted into the blood to regulate the free hormone levels (Litwack, 2018). It modulates the bioavailability of these hormones and ensures their controlled release to target tissues. The potential impact of CBG levels on cortisol availability, particularly in the stress response during the acute phase, underscores the urgency of understanding this process (Cizza and Rother, 2012).

Henley and Lightman (2011) thoroughly reviewed CBG functions in humans, including its role as a transporter protein and as a temperature-sensitive protein that releases cortisol when the temperature rises. In neuroscience studies, CBG has been found in the hypothalamus and cerebrospinal fluid, suggesting a role in regulating glucocorticoid access to their receptors within the central nervous system (Henley and Lightman, 2011). Furthermore, CBG genetic variants were linked to hypotension and fatigue-pain syndrome, affecting cortisol's access to brain glucocorticoid receptors and revealing new mechanisms for regulating glucocorticoid access to the brain and tissues. Conversely, Meyer *et al* (2016) identified several mutations in the SERPINA6 gene that impact CBG expression or glucocorticoid affinity. These mutations were associated with typical clinical symptoms, including depression, headache, hypotension, fatigue, chronic pain, obesity and irregular hypothalamic-pituitary-adrenal axis activity.

In humans, CBG has been extensively studied; however, there are no specific direct investigations into the role and characteristics of CBG in camels. A recent study in dromedary camels linked increased cortisol levels and adrenocortical activity to the clinical observations, post-partum changes and

milk-serum antioxidant markers as indicators of stress (Khalfallah *et al,* 2024). This physiological shift suggests that CBG levels may also rise to buffer the cortisol surge and maintain hormonal balance. Furthermore, the study also identified notable interactions between cortisol and oxidative stress biomarkers, which may highlight the role of CBG in regulating stress responses during the critical physiological transition period.

Consequently, there is an urgent need to study camel CBG, given their unique adaptations to heat stress and dehydration. Accurately measuring CBG levels in camels is crucial for distinguishing between free and bound glucocorticoids, as it aligns with CBG binding, which significantly impacts circulating, free and active cortisol levels. Free cortisol levels are key indicators of physiological stress and metabolic adaptation to extreme conditions. The available physiological evidence and comparative endocrinology studies in humans and other animal species indicate that CBG plays a vital role in the regulation of glucocorticoid hormones and stress adaptation. Future research should concentrate on the molecular characterisation and regulatory mechanisms of camel CBG, which will deepen our understanding of camelid endocrine physiology and pave the way for potential practical applications in this field.

The clinical significance of APPs in camels

The data from this review offers a deep understanding of the behaviour of APPs in camels. This knowledge is both critical and directly applicable to managing their health. In clinical medicine studies, APPs are often utilised as practical tools and clinical biomarkers for detecting and monitoring disease progression. Their theoretical role, especially in comparative immunology research, is fascinating and engaging. The review discusses the assessment of APPs levels and uses practical methods to improve camel health management by identifying subclinical conditions that may not be visible through clinical examination alone. Notably, positive APPs are being studied and actively applied as early biomarkers for inflammation and infection in camel diseases, providing valuable information for timely diagnosis and treatment. The review also indicates that regular monitoring of positive APPs levels in camels is not just a suggestion; it is a practical approach that can help track disease progression and assess the effectiveness of treatments. This proactive approach significantly enhances understanding of immune responses across different species, contributing to the development of cross-species veterinary practices.

Moreover, this review is not merely a discussion; it is a comprehensive analysis that thoroughly addresses the behaviour of negative APPs in camels, emphasising their crucial role for various reasons, such as detecting nutritional status and metabolism, assessing liver function and identifying inflammatory and stress biomarkers, along with disease diagnosis and management. Understanding and applying this knowledge can significantly benefit the health and welfare of camels, offering hope for improved disease diagnosis and management. Since the liver produces many APPs, variations in their levels can provide insights into liver function and health. Albumin, transferrin and other negative APPs act as indicators of nutritional status and their levels can assist in evaluating camels' welfare, health and dietary requirements. A decrease in negative APPs can complement the rise in positive APPs, offering a more holistic view of the animal's inflammatory status.

Therefore, monitoring both camel's positive and negative APPs can improve the accuracy of disease diagnosis, facilitating better management and treatment of infectious and inflammatory diseases. Promising research into AT, TTR, RBP and CBG levels is shedding light on changes in stress hormone metabolism and stress biomarkers, a vital area of study for understanding how camels cope with stressors such as transportation, climate change and emerging diseases. This approach can help veterinarians and researchers develop more effective strategies to ensure the health and welfare of camels, particularly under stressful or disease conditions. Studying the acute phase response in camels can also provide valuable information for comparative immunology and help improve health management practices across different animal species. The application of advanced techniques, such as proteomics and genomics, can further elucidate the regulation and function of negative APPs, leading to improved diagnostic and therapeutic approaches and instilling hope for the future of camel health.

In conclusion, the APPs hold great potential as biomarkers for monitoring health and disease in camels. However, current knowledge remains limited and mainly derived from other animal species. Considering the camel's unique immunophysiological adaptations to arid environments, targeted research is urgently needed. Future studies should establish camel-specific baseline reference ranges and evaluate

the influence of age, sex, breed and reproductive status on APP levels. It is equally essential to characterise APP dynamics in major camel diseases such as trypanosomosis, respiratory infections, mastitis and parasitic infestations, with a focus on their utility for detecting early or subclinical cases. Additionally, assessing the diagnostic sensitivity, specificity and prognostic value of APPs in camels compared to conventional haematological, biochemical and immunological biomarkers will help clarify their role in monitoring treatment responses and welfare under environmental stress. Advancing these studies will significantly improve disease detection, prognosis, herd health management and evidence-based veterinary care, ultimately enhancing the productivity, welfare and resilience of camels in arid and semi-arid regions, where they play a critical socio-economic role.

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ASSESSMENT OF CAMEL FEED RESOURCES: WOODY SPECIES STAND STRUCTURE, SPECIES RICHNESS AND DIVERSITY IN TSABONG ECOTOURISM CAMEL PARK, SOUTH-WESTERN BOTSWANA

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ABSTRACT

Stand structure, diversity and regeneration status of woody species, were studied in Tsabong Ecotourism Camel Park (TECP), south-western Botswana. Regeneration status of the woody species, stand structure i.e., population structure, density, abundance, frequency, dominance, richness, diversity and evenness and important value index (IVI) were determined by genera and family, we used 40 quadrats of 20 m × 20 m placed systematically within TECP. There were 11 woody species in total, from 5 families and nine genera. The woody species exhibited a diversity of 2.1 and an evenness of 0.6. The average density of the woody species was 3,585 individuals ha^{-1} and varied from 768 to 19 individuals ha^{-1}. In terms of density, the five woody species with the highest density (≥ 100 individual ha^{-1}) were *Senegalia mellifera, Elephantorrhiza elephantina, Rhigozium trichtomum, Vachellia karoo and Vachellia erioloba*. Woody species occurred with frequencies ranging from 7 to 78%. The most frequently recorded woody species were *V. erioloba, S. mellifera, E. elephantina, G. flavescens and S. italica*. The dominance of woody species varied from almost 0 to 4 ha^{-1}. The dominant species included *V. erioloba, B. albitrunca* and *V. karroo*. Nine of the species exhibited dominance values of 0 or 1 $0ha^{-1}$. The ecological importance of the woody species is represented by the IVI, which ranged from 3 to 89%. The woody species with an IVI of ≥ 20%, ranked by ecological importance from highest to lowest, were *V. erioloba, S. mellifera, E. elephantina, V. karoo* and *R. trichotomum*. The population structures of only 27% of the woody species were stable, whereas most woody species (73%) showed unstable population structures, with hindered natural regeneration. Several potential research directions were presented with suggestions for the future sustainable management of TECP.

Key words: Botswana, camel feed, diversity, ecotourism, feed resources

The identification of feed resources for camel production relies on assessing the richness, diversity and regeneration status of woody plant species. Camels' diet and sustainable milk production greatly rely on the valuable contribution of woody vegetation resources.

In Botswana, camels (*Camelus dromedarius*) are kept in Tsabong, which is a semi-arid region in Kgalagadi District. They are kept in an exclosed park known as Tsabong Ecotourism Camel Park (TECP). Camels are kept in Tsabong mainly for tourism, specifically for riding purposes. Nevertheless, the camels are utilised for milk and meat production, but these products are exclusively consumed by the park employees and have not been made available for sale in the market yet.

A better equitable distribution of individuals of different woody species in the TECP is expected due to the low natural and anthropogenic disturbances since the area is fenced to exclude livestock and humans. Livestock grazing and browsing pressure affect species richness and recruitment of plant communities (Jacobs and Naiman, 2008; Levick and Rogers, 2008). Woody species are an important component of the flora of many ecosystems in Botswana, including the TECP.

Seedling densities in forest understories are dynamic and rates may vary among species and in gap and shade environments (Bazzaz, 1991). The rates also vary due to mortality, which could include abiotic stresses, such as light, drought and biotic

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factors that include herbivory/predation, diseases or competition (Janzen, 1971; Augspurger, 1984).

The major constraint that hinders the productivity of camels in Tsabong is feed shortage especially during the dry season. The study area experiences low rainfall. This results in the area having poor pasture, making it difficult to meet maintenance nutrient requirements of camels. The area is characterised by high ambient temperature and the vegetation is dominated by sparsely distributed shrubs and trees in particular *Senegalia/Vachellia* species.

The Tsabong Ecotourism Camel Park (TECP) is an exclosure that covers a 4 x 4 km fenced area where the camel herd are kept. The camels browse inside the park all year round and they do not have access to the vegetation outside the park. Moreover, the park is not divided into paddocks and no rotational grazing is practiced in the park. As a result, the park is overgrazed and heavily degraded and dominated by only few plant species such as *Senegalia/Vachellia* and *Grewia* spp. Consequently, the natural browse species found inside the park hardly meet the nutrient requirements of camels limiting their productivity. Therefore, it is important to assess the vegetation inside TECP, analyse the factors that contribute to the degradation of the area and suggest strategies that will enable regeneration of the vegetation in the park and improve the overall feed resource base of the camel park.

The present study focuses on the assessment and identification of Camel Feed Resources in terms of woody plant species density, richness, diversity and regeneration status in TECP. Therefore, the specific objectives of the study were to: determine woody species stand structure, density, abundance, richness, diversity, frequency, dominance and important value index and to assess the regeneration status of the woody species in TECP.

Materials and Methods

Description of Study Site

The research was conducted in Tsabong, situated in the south-western part of Botswana within the Kgalagadi South District (Fig 1). The Tsabong Ecotourism Camel Park was established in 2003 and it spans an area of 16 km^2 and at present, it accommodates around 370 camels (Seifu *et al,* 2019). There are no other herbivores within the park and the park is exclusively composed of grazing land with no additional land uses.

The Kgalagadi South District is known for its hot and dry climate, experiencing summer temperatures between 28.5 and 35°C and winter temperatures ranging as low as 1 to 12°C. Rainfall is low and inconsistent, occurring mainly during the summer months from November to March reaching an average of 146.2 mm per annum (Kgaudi *et al,* 2018) and fluctuating monthly between 0.6 and 61mm (Zweistra, 2012).

Soils in the district are mostly sandy and unproductive (Batisani, 2010), which limits arable agriculture (Seifu *et al,* 2019; Tselaesele *et al,* 2021). The area is typified by sparsely distributed vegetation mostly consisting of *Grewia, Senegalia, Vachellia* and herbaceous species (Kgaudi *et al,* 2018).

Vegetation sampling

The authors established 40 sampling plots of 20 m × 20 m (each representing 0.04 ha) that were carefully established inside TECP using the Global Positioning System (GPS). A systematic random sampling method was used while establishing the sampling plots. The first plot was laid out at the north-west corner of the exclosure. Measurements were made within the sampling plots to determine the identity of species, density, abundance, population structure, frequency, dominance, richness, diversity, evenness, importance value index and regeneration status of the wood species by genera and family. In each sampling plot, the following parameters were recorded: total number of all live individuals and diameter at breast height (DBH) per woody species with DBH > 2 cm. In the case of juveniles (seedlings and coppices < 1.5 m in height), the number of individuals of each woody species was counted and recorded. A calliper and a graduated measuring stick were used to measure the DBH and height, respectively, of the woody species.

The woody species were identified directly in the field using the available literature on the flora of Botswana and Southern Africa (Timberlake, 1980; van Wyk and van Wyk, 1997; 2007; Palgrave, 2002; Setshogo, 2002; 2005; Setshogo and Venter, 2003) and with assistance from TECP officers and local communities familiar with the flora of the area. Plant nomenclature in this article follows that of Setshogo and Venter (2003), Kyalangalilwa *et al* (2013) and Setshogo (2005).

Limitation of the study

We were unable to include a control site however, we have tried to compare our findings

with results from similar studies in analogous environments, namely Mokolodi Nature Reserve (exclosed to prevent livestock grazing, but allowing wild animals to graze), Okavango Research Institute (exclosed for more than 10 years to prevent grazing of both domestic and wild animals), Maun Educational Centre (exclosed to prevent domestic animals, but allowing grazing of wild animals), Xobe and Shorobe open/free grazing sites in Botswana and similar sites elsewhere.

Data Analyses

The woody vegetation attributes (i.e., species diversity (richness, evenness), densities (DE), frequencies (FR), dominance (DO), relative densities (RDE), relative frequencies (RFE), relative dominance (RDO) and from those values, the Importance Value Indices (IVI)) were analysed using descriptive statistics.

Species richness (S) is the total number of different woody species present in the study site and does not consider the proportion and distribution of each woody species (Teketay *et al,* 2018). Woody species richness in the TECP was evaluated using the total number of different species recorded in all the quadrats. The diversity of woody species was analysed using the Shannon Diversity Index (H') (also referred to as the Shannon-Weiner/Weaver Diversity Index in Ecological literature) (Krebs, 1989; Magurran, 2004). The index considers the species richness and the proportion of each woody species in all the sampled quadrats (Teketay *et al,* 2018). The woody species diversity was analysed using the following formula:

$$H' - \sum_{i=1}^{s} Pi \ln Pi$$

where, H' = Shannon index, S = species richness, P_i = proportion of S made up of the i^{th} species (relative abundance).

Evenness or equitability, a measure of similarity of the abundance of the different woody species in the study site was analysed by Shannon's Evenness or Equitability Index (E) (Krebs, 1989; Magurran, 2004). Evenness values range from 0 to 1, with 1 being complete evenness and calculated using the following formula:

$$E=H'/\ln S$$

where, E = evenness and S = species richness.

The mean density of each woody plant species (ha^{-1}) in the TECP was determined by converting the total number of individuals of each woody species recorded in all the quadrats to an equivalent number ha^{-1}.

The frequency was calculated as the proportion (%) of the number of quadrats in which each woody species was recorded from the total number of quadrats within TECP.

The dominance of the woody species, with DBH of ≥ 2 cm, was determined from the space occupied by a species, i.e. its basal area (BA). It was computed by converting the total BA of all the individuals of each woody species to equivalent BA ha^{-1} (Kent, 2012).

The Importance Value Index (IVI) indicates the relative ecological importance of woody plant species in the sampled areas (Kent, 2012). It was determined by summing up the relative values of density, frequency and dominance of each woody species. The species with the highest IVI were considered the most ecologically important in the study area (Ismael *et al,* 2017). Relative density was calculated as a percentage of the density of each species divided by the total stem number of all woody species per hectare (Teketay *et al,* 2018). Relative frequency was computed as the ratio of the frequency of the species to the sum of the frequency of all species encountered in TECP. Relative dominance was calculated as the percentage of the total basal area of a species out of the total basal areas of all species. IVI was used to compare the overall dominance and ecological significance of species.

The population structure and regeneration status of each woody species in the study area were assessed through histograms constructed by using the density of individuals of each species (Y-axis) categorised into 10 diameters classes (X-axis) (Peters, 1996), i.e.: 1 = < 2 cm; 2 = 2-5 cm; 3 = 5-10 cm; 4 = 10-15 cm; 5 = 15-20 cm; 6 = 20-25 cm; 7 = 25-30 cm; 8 = 30-35; 9 = 35-40; 10 = > 40 cm. Thereafter, based on the profile depicted in the population structures, the regeneration status of each woody species was determined (Teketay *et al,* 2018).

Results

Species Richness, Diversity and evenness

A total of 11 different woody species, representing five families and 9 genera were recorded in TECP (Table 1). Of these, one species was unidentified. The most diverse family, Fabaceae (Leguminosae), had six (about 55%) woody species. One species (roughly 9% of each) represented each of the other families (Bignoniaceae, Brassicaceae,

Tiliaceae, Thymelaeaceae, and unidentified species) (Table 1).

Vachellia, represented by three species (= 27%), was the genera with the highest richness of woody species. Each of the remaining genera (*Arthrosolen, Boscia, Elephantorrhiza, Commiphora, Grewia, Rhigozum, Senegalia, Senna* and unidentified species) was represented by a single species, accounting for 9% each. The woody species diversity (H') and evenness (E) in the TECP were recorded as 2.1 and 0.6, respectively.

Woody Species Density, Frequency and Dominance

The total mean density of the woody species across the study area was 3,585 individuals ha^{-1} (Table 1) and ranged between 768 (*S. mellifera*) and 19 individuals ha^{-1} (*B. albitrunca*). The most densely populated woody species, in descending order, were *Senegalia mellifera, Elephantorrhiza elephantina, Rhigozium trichtomum, Vachellia karoo, Vachellia erioloba, Logolo* (unidentified) *Senna italica, Arthrosolen polycephalus and Grewia flavescens. Vachellia hebeclada and Boscia albitrunca* were the least densely populated woody species in the study area (Table 1).

The frequency of occurrence of woody species ranged from 7% to 78% (Table 1). The top 5 most frequently recorded woody species were *V. erioloba, S. mellifera, E. elephantina, G. flavescens and S. italica.* However, *A. polycephalus, R. trichotomum, Logolo, V. hebeclada and B. albitrunca* were among the less frequently recorded woody species.

Dominance of the woody species in the study area varied from 4 to nearly 0 individuals ha^{-1} (Table 1). The dominant species based on basal area were *V. erioloba, B. albitrunca and V. karroo*. Other species had minimal contributions to total basal area, each between 0.0 and 0.1 m^2/ha.

The Importance Value Index (IVI) of the woody species varied from 3% to 89% as shown in Table 1. The woody species with the highest IVI (≥ 20%) in descending order of ecological importance were *V. erioloba, S. mellifera, E. elephantina, V. karoo and R. trichotomum. On the other hand, V. hebeclada and Logolo* had IVI values below 10%. 10%.

Population structure and regeneration status of woody species

The woody species recorded in the study area were categorised into three groups based on their population structures as follows:

I. The first pattern exhibited the highest number of individuals at the lowest diameter class and progressively declining numbers with increasing diameter classes. This group of species was represented by *V. karoo, S. mellifera* and *V. erioloba* (representing 27% of the total number of species) (Fig 2).

II. The second pattern was formed by woody species which exhibited a similar diameter class distribution pattern as the first group except that individuals at the higher diameter classes are missing. This group was represented by *B. albitrunca* (Fig 2) (representing 9% of the total number of species).

III. The third pattern exhibited woody species with individuals only in the lower diameter classes (Fig 2). This pattern was represented by *V. hebeclada, E. elephantina, G. flavescens, R. trichotomum* Burch., *Logolo* (unidentified sp.) and *Arthrosolen polycephalus*.

Discussion

Forest and woodland resources in Botswana provide goods (timber, food fuelwood, medicine, etc.) and services (soil protection carbon sequestration, wildlife habit and regulation of water resources) that are important in the sustenance of livelihoods and the economy at large. Woody species are an important component of the flora of many ecosystems in Botswana, including the TECP.

Woody species richness plays a crucial role in the biodiversity of forests as resource and habitat provider for almost all other species within a forest (Malik, 2014). Species richness defines the absolute number of species present in the population of interest (Aisling *et al,* 2018). Fabaceae (Leguminosae) was the most diverse family in the present study. This agrees with work done in other parts of Botswana- (Neelo *et al,* 2013, 2015; Teketay *et al,* 2016; Teketay *et al,* 2018) and other savanna ecosystems (Atkinson- and Marin-Spiota, 2014; Muluneh *et al,* 2013).

The number of species, families and genera in this site is very low compared with values reported from other woodlands in other parts of Botswana (Teketay *et al,* 2016; Neelo *et al,* 2013, 2015) and elsewhere (Bekele and Abebe, 2016; Boz and Maryo, 2020; Dibaba *et al,* 2020; Rangkuti *et al,* 2023). The family with the highest number of species recorded was Fabaceae, with known significant roles in nitrogen fixation and enhancement of soil fertility, which are important for sustaining forage.

Although, the cause of low woody species richness was not investigated in this study, it may

be attributed to browsing and grazing pressure from camels. Browsing by camels kept in the study site could reduce species richness by direct consumption and trampling of seedlings and saplings. Sustained browsing pressure kills woody species (Levick and Roger, 2008; Staver *et al,* 2009; 2012) and prevents saplings from maturing into adults (Augustine and Decalesta, 2003; Staver *et al,* 2009). Signs of stunted growth and canopy loss were observed in the woody species, which may indicate browsing pressure exerted by camels. Furthermore, the study site is in a low rainfall area (146.2 mm per annum) (Augustine and Decalesta, 2003) and on nutrient-poor sandy soils. Therefore, the sandy soil and insufficient moisture may be affecting woody species richness. Rainfall is an important determinant describing woody vegetation communities and low rainfall limits primary productivity (Sankaran *et al,* 2005; Ward, 2005; Kraaij and Ward, 2006).

The Shannon diversity index value recorded for this study area is like those reported for Shorobe (2.18) Island Safari Lodge (2.16) (Neelo *et al,* 2013, 2015) and inside the Okavango Research Institute compound (ORI) [(Teketay *et al,* 2018) in Botswana. In contrast, the Shannon diversity index recorded in the study site is higher than those outside the ORI compound (1.6) Mokolodi Nature Reserve (1.44) (Teketay *et al* 2016) in Botswana. These results show that woody species in the site are more diverse, though a few species were recorded. This could be attributed to the less dominance of a few species over the others, due to low habitat disturbance and environmental conditions that favour all species (Zegeye *et al,* 2006; Tadele *et al,* 2014).

The Shannon diversity index reflects the level of diversity (Susilowati *et al,* 2019) and stability (Wang *et al,* 2020) of plant communities within a site. A diversity value of 1.0 is considered very low (Zegeye *et al,* 2006).

Species evenness index (E) indicates the relative abundances of species within a community (Wilson and Witkowski, 2003; Cavalcanti and Larrazabal, 2004; Susilowati *et al,* 2019). The evenness value (0.60) recorded in this study is close to those reported inside (0.75) and outside ORI (Teketay *et al,* 2018) Xobe (0.5), Shorobe (0.6) (Neelo *et al,* 2013, 2015) in Botswana and elsewhere (Zegeye *et al,* 2011; Worku *et al,* 2012). Results suggest that there is a better equitable distribution of individuals of different woody species in the TECP. This could be attributed to the low natural and anthropogenic disturbances since the area is fenced to exclude livestock and humans. Livestock grazing and browsing pressure affect species richness and recruitment of plant communities (Jacobs and Nainan, 2008; Levick and Rogers, 2008).

The mean density of plants reported in this study (3,585 individuals ha^{-1}) is lower than that of Shorobe (Neelo *et al,* 2013), Mokolodi Nature Reserve (Teketay *et al,* 2016) and Maun Educational Centre but greater than that of Xobe (Neelo *et al,* 2013. The density of woody species in TECP ranged from 19 to 768 individuals ha^{-1}. *Senegalia mellifera, Elephantorrhiza elephantina* and *Rhigozum trichotomum* exhibited the highest mean density.

Results showed that 5 species viz *V. erioloba, S. mellifera, R. trichotomum, G. flavescens* and *S. italica* were the most frequent in this site. Which contributed over

Table 1. List of woody species recorded in Tsabong Camel Park with their families, densities (DE = individuals ha^{-1}), frequencies (FR), dominance (DO), relative densities (RDE), relative frequencies (RFE), relative dominance (RDO) and important value indices (IVI).

Species		Family	DE	FR	DO	RDE	RFR	RDO	IVI
1.	*Senegalia mellifera*	Fabaceae	768	78	0.09	21	17	14	53
2.	*Elephantorrhiza elephantina*	Fabaceae	694	65	0	19.4	15	0	34
3.	*Rhigozum trichotomum*	Bignoniaceae	676	10	0	19	2	0	21
4.	*Vachellia karroo*	Fabaceae	277	30	0.94	7.7	6.7	15	30
5.	*Vachellia erioloba*	Fabaceae	255	85	4	7	19	62	89
6.	*Logolo* (Setswana name)		243	8	0	7	2	0	8.4
7.	*Senna italica*	Fabaceae	229	55	0	6.4	12	0	19
8.	*Arthrosolen polycephalus*	Thymelaeaceae	219	38	0	6	8	0	15
9.	*Grewia flavescens*	Tiliaceae	152	65	0	4	15	0	19
10.	*Vachellia hebeclada*	Fabaceae	52	8	0	1.2	2	0	3
11.	*Boscia albitrunca*	Capparaceae	19	7	1	1	2	8	10
			3,585	447	6				300

Fig 1. Map of Africa showing the location of Botswana Source: https://maps-bostwana.com/botswana-africa-map (accessed on 281224) and study area, Tsabong village (Source: Seifu *et al,* 2019).

78% of the cumulative frequency recorded for all the species. A high frequency indicates a wider distribution of species on the site (Teketay *et al,* 2018). According to Gedefaw and Soromessa (2014), frequency provides an approximate indication of the homogeneity and heterogeneity of vegetation in a community. A high frequency indicates a wider distribution of species on the site (Teketay *et al,* 2018). A high value in higher frequency and a lower value in the lower frequency classes in a site indicate heterogeneity of vegetation (Lamprecht, 1989; Shibru and Balcha, 2004).

Dominance was evaluated using basal area, which is an important parameter for measuring the relative importance of the woody species than simple counting of stems (Aliyi *et al,* 2015). Woody species with the largest contribution to the overall basal area in each community are considered the most important in a habitat (Bekele, 1994; Aliyi *et al,* 2015; Endris *et al,* 2017). In this study, *Vachellia erioloba* was the most dominant and important woody species (IVI = 89%, Table 1) with a basal area of 4 m^2 ha^{-1} (67%) which is about four times than that of the second most important species with a basal area of approximately 1 m^2 ha^{-1}. It is also one of the species with the highest mean densities (259 individuals ha^{-1}) in TECP (Table 1). Approximately 73% of the woody species in this site lacked trees with high DBH values and their basal area ranged between 0.0 - 0.1 m^2 ha^{-1}. This could be attributed to poor environmental conditions (moisture deficiency, poor soils and frost) and pressure from camel browsing.

Importance Value Index is an important parameter used to compare the ecological significance of species in a vegetation community (Lamprecht, 1989; Zegeye *et al,* 2006; Dirbaba *et al,* 2014; Gedefaw and Soromessa, 2014; Tilahun *et al,* 2014; Ayanaw and Dalle, 2018; Pamoengkas *et al,* 2019) and is derived from a combination of data from relative frequency, relative density and relative dominance (Kent, 2012). Woody species having the highest IVI is considered dominant in a site (Arshad *et al,* 2002; Noraimy *et al,* 2014) and more important than their counterparts with low IVI (Eyasu *et al,* 2020; Noraimy *et al,* 2014). The results revealed that *Vachellia erioloba, Senegalia mellifera, Elephantorrhiza elephantina, Vachellia karroo* and *Rhigozum trichotomum* with IVI values of more than 20 are the leading dominant and ecologically most significant woody species in TTCP. The dominant and ecologically most significant species could also be the most successful species in regeneration in the site, tolerate sandy soil, low rainfall and resist browsing by camels. Moreover, the dominant woody species belong to the Fabaceae family with some members known to have nodules formed by rhizobia, which can fix nitrogen available in the air into an organic form for their use, which helps them to grow well in the present study site characterised by sandy soils.

Tree size class distribution is an important indicator of changes in population structure and species composition of a forest ecosystem (Condit *et al,* 1998; Neelo *et al,* 2015). Population structure of woody species yields information on the history

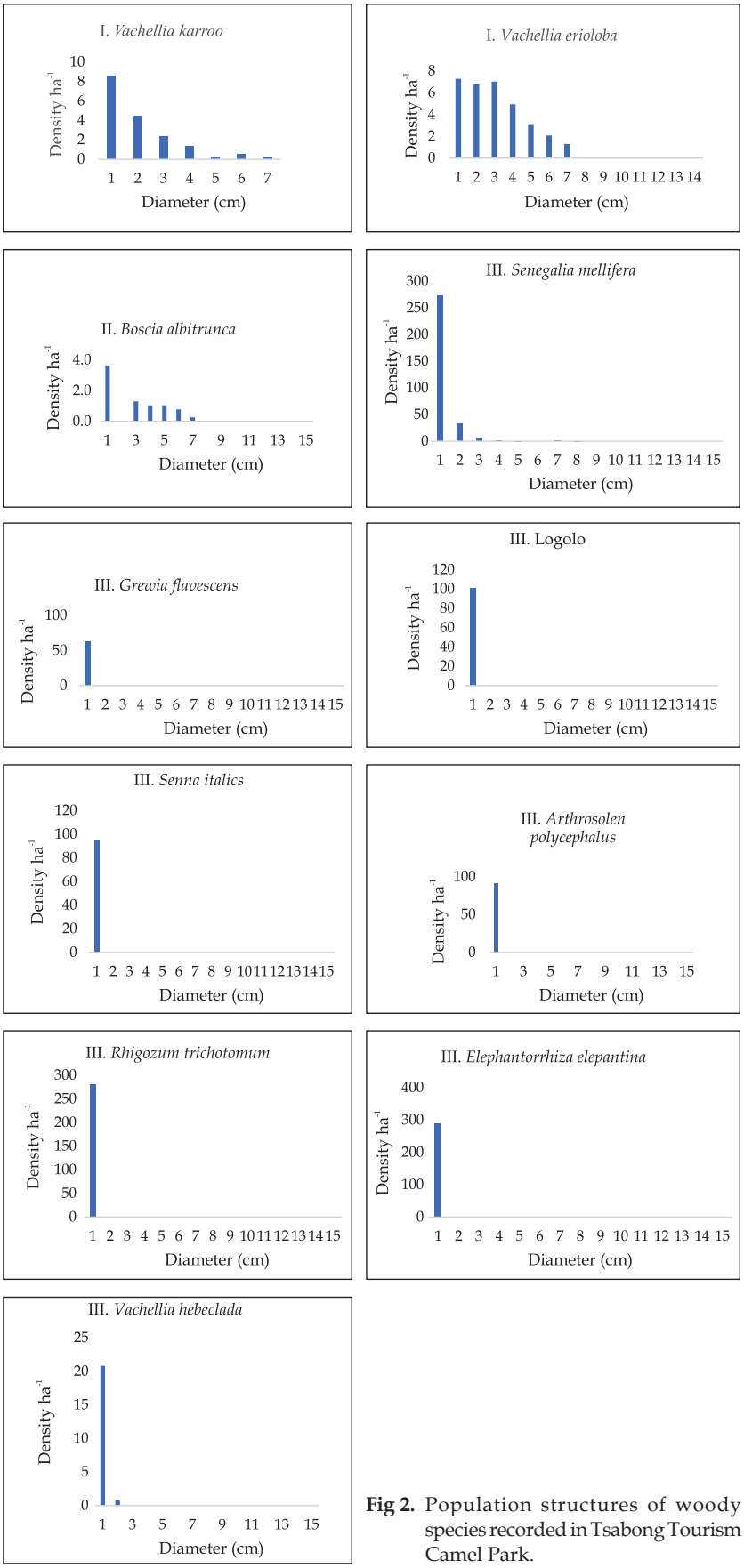

Fig 2. Population structures of woody species recorded in Tsabong Tourism Camel Park.

of past disturbance of the species and their environment (Teketay, 1997b; Wale *et al,* 2012; Neelo *et al,* 2015), which can be used to predict the future trend of the population of a particular species (Teketay, 1997a; Wilson and Witkowski, 2003; Kalema, 2010; Neelo *et al,* 2015).

The assessment of diameter class distributions of woody species in TECP resulted in the recognition of three different patterns of the population structures. In the first group, to which only about 27% of the woody species belong, the number of individuals decreased with the increasing diameter class, resulting in an inverted J-shaped population, an indication of stable population structure or healthy regeneration status (Teketay, 1997a; Alelign *et al,* 2007; Tesfaye *et al,* 2010; Zegeye *et al,* 2011; Neelo *et al,* 2015; Teketay *et al,* 2016). The woody species (about 73% of the woody species), which were categorised in the two other groups of population structure exhibited hampered regeneration, suggesting that the vegetation in TECP has been highly degraded because of a long period of open grazing/overgrazing. Human disturbance, particularly grazing, has been reported as the major reason for hampered or poor regeneration (Zegeye *et al,* 2011; Neelo *et al,* 2013, 2015). High browsing pressure can lead to the absence of seedlings or juveniles because of high seedling mortality (Trembley *et al,* 2007; Negussie *et al,* 2008; Neelo *et al,* 2013, 2015).

The population structures of woody species recorded in TECP were similar to those reported for Mokolodi Nature Reserve, which had a history of serving as a ranch and is, currently, being used as a habitat for grazing by various wild animals (Teketay *et al,* 2016).

Among the woody species that were categorised in the first

group, which exhibited healthy regeneration, are *B. albitrunca, V. erioloba, S. Mellifera and V. karoo,* which are among the relatively densest (Table 1) and preferred browse species by the camels. Despite their healthy regeneration exhibited by their population structures (Fig 2), these species lacked individuals at the bigger diameter classes, which represent the adult and reproductive individuals. On the other hand, the woody species categorised in the third group, e.g. *Grewia flavescens, R. Trichocomum, V. hebaclada, Senna italica, Arthrosolon policephalus E. elephantia* and *Logolo* (Unidentified sp.) are bound to local extermination or disappearance since they are represented by individuals only at the lowest diameter classes, i.e. juveniles (seedlings and coppices) (Fig 2). Hence, they require attention in the active management of the woodland in TECP.

Nine browse species consumed by camels were identified in the park of which *Boscia albitrunca* is the browse species most preferred by the camels (Kgaudi *et al,* 2018). The result may explain the relatively low mean density (19 individuals ha^{-1}) of the species in TECP (Table 1).

In a study undertaken to determine the major browse species consumed by dromedary camels in Tshabong (Kgaudi *et al,* 2018), 9 species of plants were reported by respondents as being the major sources of feed in TECP. The nine species belonged to 7 families and 7 genera of flowering plants. Fabaceae and *Vachellia* exhibited the highest proportion 33.3 and 22.2%, respectively of the 9 browse species. This is also consistent with results from the present study in which Fabaceae (55%) and *Vachellia* (27%) showed the highest number of species. Of these, four of the woody species, namely *B.albitrunca, R. trichotomum, S. mellifera* and *V. erioloba,* have been recorded in the current study. Three of these species, namely *B. albitrunca, S. mellifera* and *V. erioloba* have been reported to have good forage value (Hendzel, 1981; Kgaudi *et al,* 2018). In addition, *S. mellifera* and *V. erioloba* exhibited relatively high mean densities (768 and 255 individuals ha^{-1}, relatively) in TECP (Table 1).

The forage values of the nine plant species were categorised as poor (22%), intermediate (22%) and good (56%) (Hendzel, 1981; Kgaudi *et al,* 2018). As just indicated above, the camels prefer the *B. albitrunca* tree whose foliage is evergreen, although some leaves are shed around flowering time. The mature leaves and twigs of *B. albitrunca* have a crude protein content of 9.04% (Aganga and Adolga-Bessa, 1999; Alias and Milton, 2003; Kgaudi *et al,* 2018) and are rich in vitamin A (Palgrave, 2000; Kgaudi *et al,* 2018). The leaves also contain high quantities of calcium, phosphorus, potassium and sodium, like other browse species, such as *G. flava* and *Senegalia mellifera* (Alias and Milton, 2003; Kgaudi *et al,* 2018). The roots provide a valuable food source for both animals and humans (Palgrave, 2000) although, the leaves and twigs are the preferred source of forage for livestock (Alias and Milton, 2003).

Nine browse species consumed by camels were identified in the park of which *Boscia albitrunca* is the browse species most preferred by the camels. However, the nutritive value of the major browse species consumed by the camels should be analysed and documented.

The results revealed that TECP contains a relatively low species, genera and family richness as well as diversity and evenness values of woody species. The density of woody species is relatively high, though dominated by individuals of a few species, notably *V. erioloba, S. mellifera, E. elephantia, R. trichotomum* and *V. karoo*. None of the species was recorded in all the quadrats. The basal areas (dominance) of almost all of the woody species were negligible, which indicates the absence or inadequate number of big-sized trees, which, in turn, suggests that TECP is still in the building or recovery phase after its exposure to heavy grazing and browsing impacts, especially because of over-stocking of camels with its associated over-grazing.

The woody species with the highest IVI values in TECP, which are indicative of high ecological importance, include, in descending order of ecological importance, were *V. erioloba, S. mellifera, E. elephantina, V. karoo* and *R. trichotomum*. On the other hand, *V. hebeclada*, exhibited the lowest IVI value.

Out of the 11 woody species, only 3 (about 27%) exhibited stable population structures, which is also indicative of good regeneration status while the rest (8 woody species = about 72%) showed unstable population structures, which could be attributed to their hampered regeneration. Therefore, there is a need to investigate the factors responsible for the unstable population structures and hampered regeneration of these woody species.

The woody vegetation of TECP should be managed and regulated properly by giving due attention to the enhancement of regeneration of the woody species with the hampered regeneration, especially those that are frequently visited and browsed by camels. It is recommended to introduce interventions, such as resting periods, rotational grazing or exclusion zones to allow species to mature.

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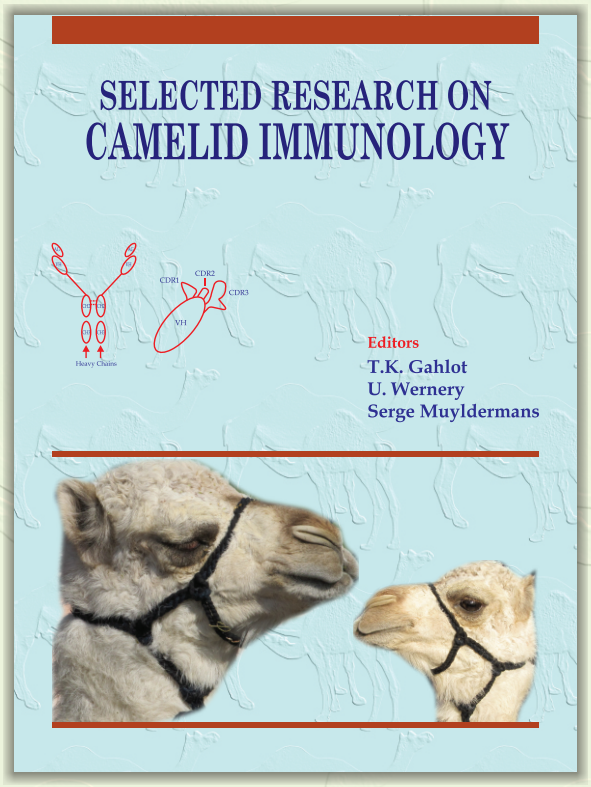
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

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

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



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MY JOURNEY TO CAMEL SCIENCE: FROM FOUNDATIONAL FIELD WORK TO HIGHLY SPECIALISED MOLECULAR RESEARCH

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ABSTRACT

My journey into camel science began in September 1990 when I joined a field mission to eastern Sudan to investigate an outbreak of a skin disease in camels. I worked in a French Sudanese Camel Research Project and studied the husbandry and production parameters of camels in the Butana area of eastern Sudan. I was awarded a research grant from the International Foundation for Science (IFS) to fund my Ph.D. research on pox and pox-like diseases in camels. Later in my post-doctoral training,with Alexander von Humboldt Fellowship in Germany from 2001 to 2003 under the guidance of Professor Matthias Buetner, I learnt molecular techniques. In January 2010, I accepted a new position in ACSAD in Syria. This role expanded my expertise in the field of camel development, including production and marketing in Sudan, Algeria, and Morocco. Later, I joined King Faisal University (KFU) in Saudi Arabia in July 2012 and established a research laboratory at the Camel Research Centre that focused on using advanced molecular techniques to diagnose and differentiate camel diseases. Our research aimed to develop multiplex PCR for rapid disease diagnosis, detect MERS-CoV, phylogenetic analysis of camel contagious ecthyma virus, and identify pathogens associated with reproductive health issues in both male and female camels. In July 2016, I moved to the Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), where I established a virology laboratory with a BSL-3 facility and implemented specialised diagnostic tests for camels. One of my significant accomplishments at ADAFSA was leading capacity-building and research activities that led to the World Organisation for Animal Health (WOAH) designating our veterinary laboratories as a Collaborating Centre for Quality Management in Veterinary Laboratories in 2020 and as a collaborating centre for camel diseases in 2021. Our center's mission remained to monitor emerging infectious diseases affecting camel health and their zoonotic potential throughout camel-raising countries. The culmination of my decades of work is showcased in the 2021 book- Infectious Diseases of Dromedary Camels, which I co-authored with the late Professor Mansour Hussein. My journey exemplifies how a scholar's work can expand in scope while deepening in scientific merit, ultimately leaving a lasting legacy in the field of camel science. International leadership I have taken on a prominent leadership role in international scientific Organisations, utilising my extensive expertise to influence global policy and advance research in veterinary medicine. My contributions span some key Organisations, with a particular focus on animal health, disease control, and the study of camelids. I have demonstrated my leadership skills by progressing to a key position within the PPR Global Research and Expertise Network (PPR-GREN), a collaborative initiative of the FAO and WOAH, and I was first elected as a bureau member in 2021. I served as Secretary-General of ISOCARD from 2009 to 2012 and later as Chairman from 2012 to 2015, during which I played a crucial role in advancing the scientific understanding of camelids.

Key words: Camel science, diseases, molecular diagnostic techniques

After graduation, I earned a master's degree in virology from the Faculty of Veterinary Medicine, University of Khartoum. I began my career as a veterinary officer in northern Sudan in November 1986. Three years later, in May 1989, I moved to the Central Veterinary Research Laboratories (CVRL) in Khartoum, where I focused on diagnosing animal diseases. During this time, I gained practical experience in essential laboratory techniques, including histopathology, electron microscopy, serological diagnosis, and cell culture, which are fundamental to traditional laboratory methodologies.

My journey into camel science began in September 1990 when I joined a field mission to eastern Sudan to investigate an outbreak of a skin disease in camels. This was my first time working with these remarkable animals. When we returned to the lab, we analysed the samples and diagnosed the disease as contagious ecthyma. The diagnosis was confirmed by detecting the characteristic viral

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particles under an electron microscope, and the results of our findings were published in my first contribution to camel health research (Khalafalla *et al,* 1984). Yet, it wasn't just the science that captivated me; it was the profound experience of working with these gentle creatures and their generous herders that truly inspired my future path.

Diving deeper into camel research

At that time, a collaborative research project was underway between the Faculty of Veterinary Medicine at the University of Khartoum and the French agricultural research and international cooperation Organisation, CIRAD. This initiative was known as the French Sudanese Camel Research Project. Al Showak city, located at 14°03' N and 35°08' E, approximately 480 km east of Khartoum, served as the project's field station. The project was aimed to study the husbandry and production parameters of camels in the Butana area of eastern Sudan (Agab, 1993). From 1991 to 1993, we visited fifteen camel herds monthly as part of this initiative. During this period, I was awarded a research grant from the International Foundation for Science (IFS) to fund my Ph.D. research on pox and pox-like diseases in camels.

The series of papers extracted from the PhD thesis was groundbreaking because it marked the first formal isolation and characterisation of the camelpox virus in Sudan. It established a crucial diagnostic baseline using classical virological techniques and provided the first field-based epidemiological study of pox and pox-like diseases in camels (Khalafalla and Mohamed, 1996; Khalafalla *et al,* 1998a; Khalafalla *et al,* 1998b; Khalafalla and Mohamed 1998c; Khalafalla *et al,* 1998d; Khalafalla and Mohamed, 1997).

The ongoing citations of this seminal work in contemporary literature, along with its impact on advancing the lead author's career in the study of emerging zoonoses, highlight its lasting influence. The true value of this research lies in its historical significance and the catalytic effect it has had on subsequent scientific inquiry and professional development.

A Teaching Career and International Collaboration

Upon completing my Ph.D. in 1997, I began a new chapter as a lecturer at the Faculty of Veterinary Medicine at the University of Khartoum. Over the next decade, I advanced from Assistant Professor to Associate and finally to Full Professor in 2004. During this time, I also took on leadership roles, serving as the Head of the Department from 2004 to 2009 and as the Director of the Camel Research Centre at the university.

A pivotal experience in my career was my Alexander von Humboldt Fellowship in Germany from 2001 to 2003. This post-doctoral training, under the guidance of Professor Matthias Buetner, equipped me with essential molecular techniques that would become central to my research.

My time at the university wasn't limited to teaching. My postgraduate students and I conducted groundbreaking research on viral respiratory infections in camels, leading to the first laboratory identification of several viruses, including parainfluenza virus 3 (Intisar *et al,* 2009), respiratory syncytial virus (RSV) (Intisar *et al,* 2010a), pestivirus (Intisar *et al,* 2010b), and adenovirus type 3 (Intisar *et al,* 2010c). This work spurred further scientific inquiry in the field.

From research to global development and policy

In January 2010, I accepted a new position as a camel development and health expert at the Arab Centre for Studies on Arid Zones and Dry Lands (ACSAD) in Syria. This role expanded my expertise beyond animal health to the broader field of camel development, including production and marketing. I conducted feasibility studies for camel ranches and led a project to improve camel milk production in Sudan, Algeria, and Morocco. I also oversaw animal health and transboundary disease projects, establishing health programs and conducting routine examinations of livestock.

Due to the civil conflict that began in Syria in 2011, I relocated to King Faisal University (KFU) in Saudi Arabia in July 2012. At KFU, I established a research laboratory at the Camel Research Centre that focused on using advanced molecular techniques to diagnose and differentiate camel diseases. Our research aimed to develop multiplex PCR for rapid disease diagnosis (Khalafalla *et al,* 2015a), detect MERS-CoV (Khalafalla *et al,* 2015b), phylogenetic analysis of camel contagious ecthyma virus (Khalafalla *et al,* 2015c), and identify pathogens associated with reproductive health issues in both male and female camels (Al-Busadah *et al,* 2017; Khalafalla *et al,* 2017).

Moving to Abu Dhabi

In July 2016, I moved to the Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), where I played a vital role in enhancing their diagnostic capabilities. With the support of the

Our Land Cruiser got stuck in the mud close to Kasamor Mountain, August 1992.

Crossing the Atbara River to meet a herd of camels; from Ieft: I, the vet technician (Abdelrahman), and two camel herders.

CVRL, Khartoum, Sudan, 1989-1995

FVM, University of Khartoum, 1995-2010

BFVA, Tubingen, Germany, 2000-2001

ACSAD, Damascus, Syria, 2010-2012

CRC, KFU, KSA, 2012-2016

ADAFSA, Abu Dhabi, UAE, 2016-2025

Fig 1. Countries and workplaces where I worked from 1989 to 2025.

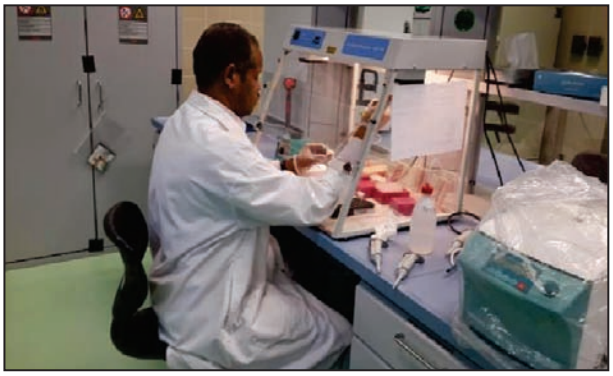
ADAFSA Director General, I established a virology laboratory with a BSL-3 facility and implemented specialised diagnostic tests for camels.

One of my significant accomplishments at ADAFSA was leading capacity-building and research activities that led to the World Organisation for Animal Health (WOAH) designating our veterinary laboratories as a Collaborating Centre for Quality Management in Veterinary Laboratories in 2020 and as a collaborating centre for camel diseases in 2021.

The recent findings of the Wesselsbron Virus Disease in camels in Ethiopia (Ishag *et al,* 2025) align with our center's mission to monitor emerging infectious diseases affecting camel health and their zoonotic potential throughout camel-raising countries.

In addition to viral diseases, our recent publications have concentrated on bacterial and parasitic infectious diseases affecting camels. These include studies on Salmonella and Theileria co-infection (Abdelwahab *et al,* 2020), tick-borne hemoparasites and *Coxiella burnetii* (El Tigani-Asil *et al,* 2021), caseous lymphadenitis (Terab *et al,* 2021), and paratuberculosis (El Tigani-Asil *et al,* 2023). Furthermore, a recent review on zoonotic diseases transmitted by camels (Khalafalla *et al,* 2023) discusses

Delivering a lecture in September 1997 at the Fac. of Vet. Medicine, U of Khartoum



Working in the safety cabinet at the Camel Research Centre, King Faisal University, December 2014

At the camel barn of the Camel Research Centre, King Faisal University, February 2013

Injecting a camel during an experiment at ADAFSA animal house, October 2021

Fig 2. Images showcasing university teaching, laboratory activities, camel barn operations, and experimental infection.

ARASCO camel workshop, December 2014, Riyadh, KSA

The 4th Conference of ISOCARD, 2015, Almaty, Kazakhstan

First International Camel Salon, Ouargla, Algeria, November 2022

Neonatal Camel Calf Diarrhoea Workshop, Al Salam Vet Group, Al Qassim, December 2024

Fig 3. Photographs depicting the delivery of opening speeches and presentations at camel workshops and conferences.

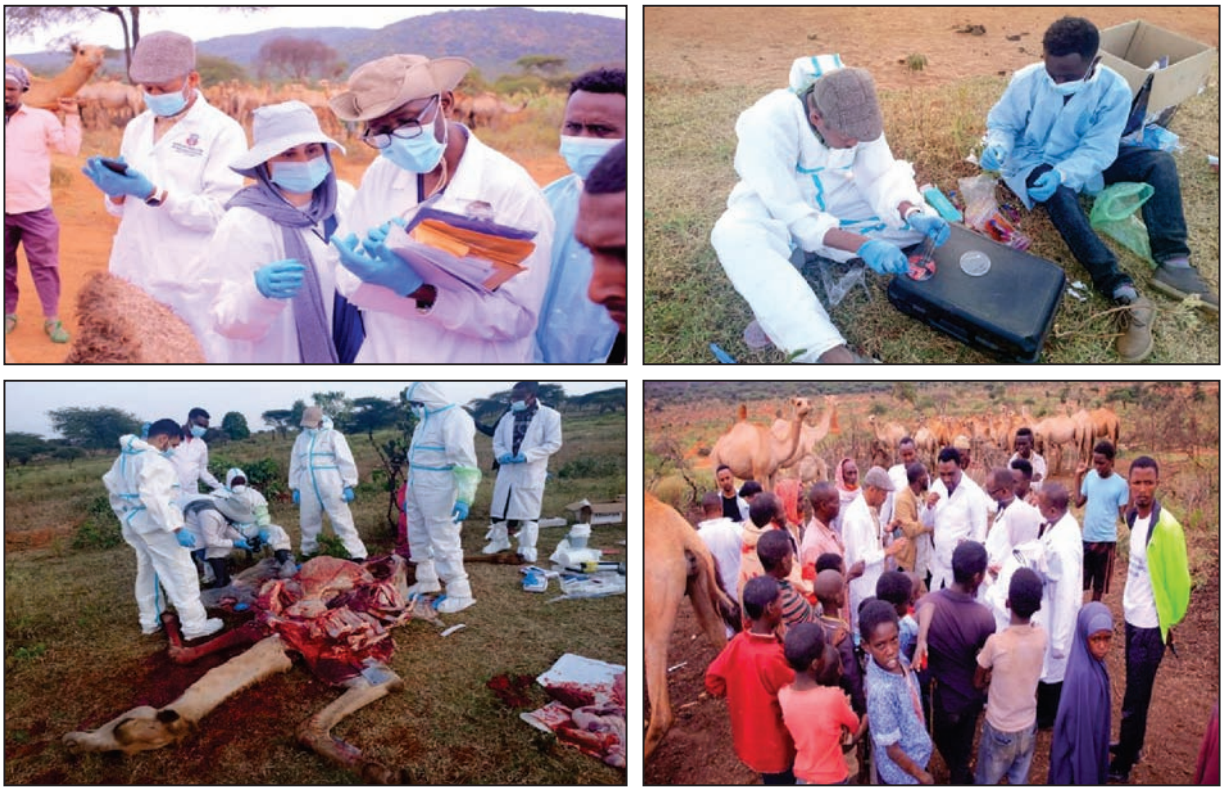


Fig 4. Photographs of ADAFSA scientists conducting a field investigation into an unknown camel disease in the Borana region of Ethiopia, July 2024.

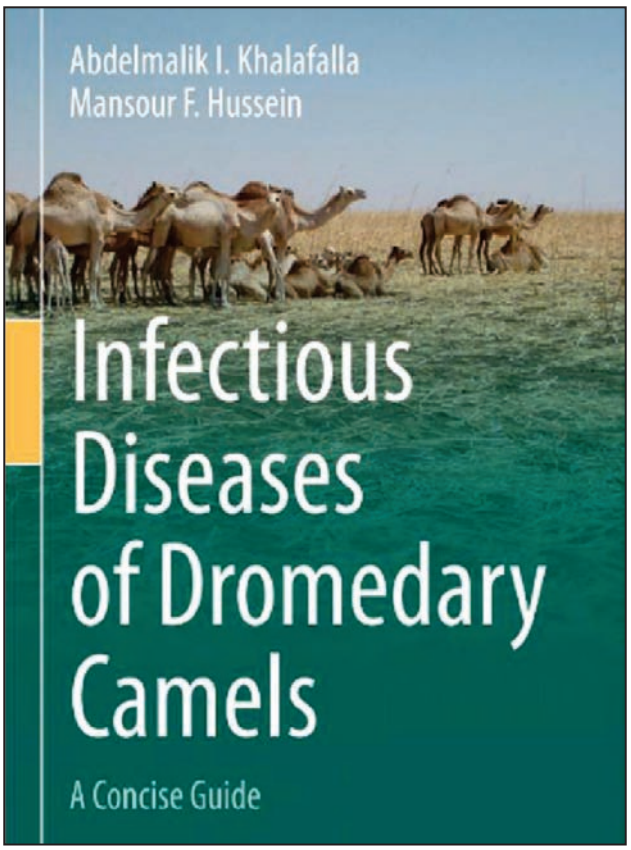


Fig 5. Cover page of our book, Infectious Diseases of Dromedary Camels, Springer Nature, 2021.

various zoonoses associated with camels, highlighting public health risks and prevention strategies that are essential for One Health approaches, which integrate epidemiology, microbiology, and public health.

From local observations to broader expertise

Throughout my career, I've seen my research evolve from studying endemic diseases in camels to exploring emerging transboundary diseases and global zoonotic threats. I began as a dedicated field researcher, documenting and characterizing diseases in underserved regions using classical methods and then advanced my work by skillfully integrating modern molecular tools. My later work on the Peste des petits ruminants virus (PPRV) in camels (Khalafalla *et al,* 2010), Middle East respiratory syndrome coronavirus (MERS-CoV) (Khalafalla *et al,* 2015b; Yusof *et al,* 2017; Paden *et al,* 2018; Khudhair *et al,* 2019; Khalafalla *et al,* 2023; Ali *et al,* 2024), and Crimean-Congo hemorrhagic fever virus (CCHFV) (Khalafalla *et al,* 2020) has directly linked camel health to public health, highlighting the enduring relevance of my foundational work. Furthermore, a recent review on zoonotic diseases transmitted by camels (Khalafalla *et al,* 2023) discusses various zoonoses associated with camels, highlighting public health risks and prevention strategies that are

essential for One Health approaches, which integrate epidemiology, microbiology, and public health.

The culmination of my decades of work is showcased in the 2021 book Infectious Diseases of Dromedary Camels, which I co-authored with the late Professor Mansour Hussein. This comprehensive volume consolidates my extensive research and that of others, serving as a definitive source of knowledge on the subject. My journey exemplifies how a scholar's work can expand in scope while deepening in scientific merit, ultimately leaving a lasting legacy in the field of camel science.

International leadership

I have taken on a prominent leadership role in international scientific Organisations, utilising my extensive expertise to influence global policy and advance research in veterinary medicine. My contributions span some key Organisations, with a particular focus on animal health, disease control, and the study of camelids. My involvement with the World Organisation for Animal Health (WOAH), formerly known as the OIE, highlights this commitment. I have served as a member of several critical ad hoc groups, including 1) Camelid Diseases, addressing the unique health challenges faced by these animals, 2) MERS-CoV, guiding effective management and control strategies for this emerging zoonotic disease and 3) Peste des Petits Ruminants (PPR) Status of Countries, advising on the management and control of this highly contagious viral disease affecting small ruminants. Additionally, I have demonstrated my leadership skills by progressing to a key position within the PPR Global Research and Expertise Network (PPR-GREN), a collaborative initiative of the FAO and WOAH aimed at eradicating this disease by 2030. I was first elected as a bureau member in 2021, and my contributions led to my election as Chair of the PPR-GREN Bureau in 2023. In addition to my work with WOAH, I have been a central figure in the International Society of Camelids Research and Development (ISOCARD). I served as Secretary-General from 2009 to 2012 and later as Chairman from 2012 to 2015, during which I played a crucial role in advancing the scientific understanding of camelids.

Appreciation words

Looking back on my journey, I am grateful to my family, mentors, supervisors, and colleagues who have supported me along the way. I began as a field researcher, documenting camel diseases in underserved regions using traditional methods. Over time, I integrated modern molecular tools into my work, allowing me to address both local animal health issues and global public health threats. My sincere gratitude goes to Prof. T.K. Gahlot for publishing this series about the paths of camel scientists in JCPR.

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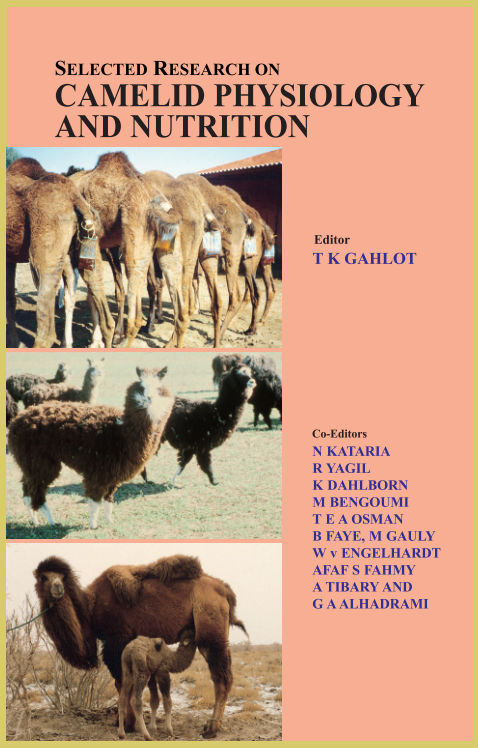

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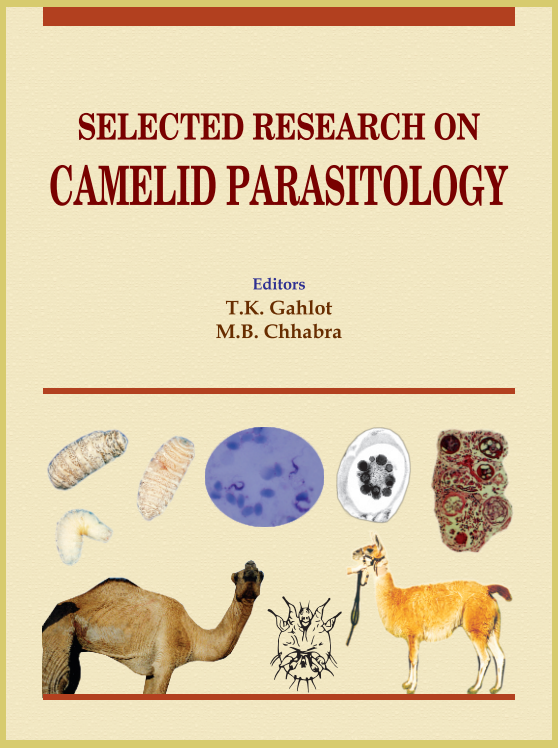

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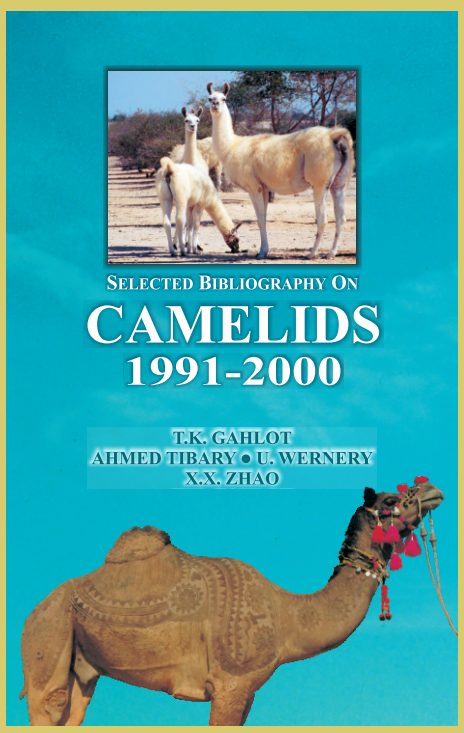

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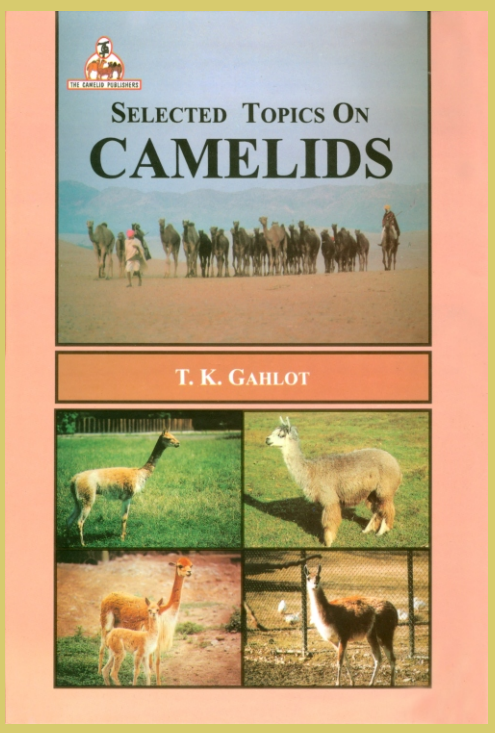

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MY JOURNEY TO CAMEL SCIENCE FROM CAMBRIDGE TO DUBAI

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ABSTRACT

My passion for adventure brought me to the Camel Reproduction Centre in Dubai where I was able to combine my love of animals with exciting research. Research as always presents many challenges, marked by periods of success and exhilaration, as well as periods of frustration and disappointment. However, whether it has been trying to relate oestrous behaviour to follicular wave patterns, working out the intricacies of embryo and semen collection, handling and cryopreservation or performing pioneering experiments hybridising New and Old World camelids it has been a time of fulfilment and satisfaction, as well as the realisation that determination and diligence can sometimes make the impossible possible! I will be forever grateful to the late Professor Roger Short who suggested I explore the world of camels, to the late Professor "Twink" Allen whose vision and encouragement has inspired me throughout my whole career and all my scientific friends and colleagues with whom I have had the pleasure of working over the years. The biology of camels will never cease to amaze me and I hope that the work we have done, and hopefully continue to do, will enable others to better understand the intricacies of camel reproduction and encourage them to explore it further.

Key words: Artificial insemination, camels, embryo transfer, embryos, semen

My first experience with camels was in 1989. At that time, I was working as a research assistant for the late Professor W.R. (Twink) Allen at the Equine Fertility Unit (EFU) in Cambridge/Newmarket when Twink was asked by His Highness Sheikh Mohammad bin Rashid Al Maktoum, now the Vice President and Prime Minister of the UAE and Ruler of Dubai, to visit Dubai to start an embryo transfer project in racing dromedary camels. I was working on embryo projects at the EFU and was therefore fortunate to be included in the project - an amazing opportunity. Together with Dr. Mike Cooper, a cattle vet from Staffordshire, we visited the Camel Reproduction Centre (CRC) in Dubai on several occasions during the camel breeding seasons of 1989 and 1990, and our exposure to these magnificent animals of the desert began.

The Camel Reproduction Centre was a small well-constructed building consisting of a camel handling area and lab space nestled amongst the sand dunes about 45 km from Dubai city and approachable only by 4WD vehicles but was further developed in 2012 to include more lab space (Fig 1). Together the three of us had several enjoyable visits to Dubai over the next two years but soon discovered that the camel held many mysteries reproductively. These we realised were going to take some time to unravel; however, we did succeed in obtaining some pregnancies from embryo transfer and, after teaching their camel vets how to recover and transfer embryos, our consultancy visits sadly drew to a close.

In 1991, whilst on a three month working trip in Australia, I met the world renowned (late) Professor Roger Short, a famous reproductive physiologist, who became a great friend and scientific mentor. After many fascinating and stimulating conversations with Roger he suggested I change from horses to camels and register for a Ph.D. in camel reproduction. I decided to take up the challenge; and it was a decision that changed my life! Thus, under the supervision of Prof Twink Allen, I registered at University of Cambridge (UK), and with the assistance of the late Dr. Billah, (Director of Zabeel Feedmill) returned to Dubai in October 1991 to start my Ph.D. entitled "Reproduction in the Dromedary Camel." It was a privilege, and a great help to me that Professor Twink Allen, Professor Roger Short and Professor Brian Heap agreed to be Governors of the CRC in those early years (Fig 2). There were many challenges in those first six months, not only with trying to unravel the intricacies of camel reproduction but also with living in Dubai (very different from the Dubai of today) as a single woman. However, with a great team at the CRC (Fig 3) these were soon overcome

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and although the initial plan was to stay three years in Dubai to complete my Ph.D., I am still here 33 years later.

For the duration of my Ph.D. (1991 – 1994), I spent the camel breeding season (October to March) in Dubai carrying out all the practical camel work and the non-breeding season (April to September) at the EFU in Newmarket doing the follow-up lab work and analysing results. As there was very limited literature published on camel reproduction we started by observing oestrous behaviour in female camels housed with a male. At the same time, we monitored ovarian follicular activity by ultrasonography and learnt that oestrous behaviour did not necessarily relate to follicular activity. We also confirmed that camels are induced ovulators, ovulating only when mated, and that follicle growth occurred in wave-like patterns with periods of growth, maturity and regression. In addition, we learnt that mature follicles capable of ovulating measured between 1.3 – 1.7 cm in diameter, but could continue to grow to as large as 4 – 6 cm if ovulation was not induced (Skidmore *et al,* 1995). We correlated this follicular activity with hormone profiles and discovered that the camel has a very short luteal lifespan of only 8 - 9 days, as evidenced by a rapid decline in serum progesterone concentrations from maximum concentrations on days 8 - 9 to basal concentrations by days 10 – 12 after ovulation. Although oestradiol concentrations increased as the follicle diameter increased, when the follicle reached 1.7 cm in diameter the oestradiol concentrations started to decline, even if the follicle continued to grow in size (Skidmore *et al,* 1996). This may account for why these large follicles do not ovulate.

We continued our studies looking at the pregnant camel and explored embryo development and placentation. We discovered that embryos enter the uterus between days 5 and 6 after ovulation as hatched, expanded blastocysts and start to elongate by days 10 – 12. As we had already concluded that luteolysis occurred around days 9 – 10 after ovulation, we tried to discover the potent maternal recognition of pregnancy (MRP) signal that passes between the embryo and maternal endometrium to signify pregnancy and inhibit luteolysis. We therefore cultured early embryonic tissue in M199 media with or without steroid precursors, (androstenedione and pregnenolone) to look for the ability of the embryonic tissue to synthesise either interferons or oestrogens. Interestingly, unlike ruminants, camel embryonic tissue does not synthesise interferon tau but, similar to the horse and pig, can synthesise oestrogens (Skidmore *et al,* 1994). This suggested that oestrogens could be involved in the MRP signal in camels. This hypothesis was further confirmed by the discovery of large, multinucleate cells at frequent but irregular intervals along the trophoblast that stained positively for the steroid- synthesising enzymes such as 3β – hydroxysteroid dehydrogenase, 17α- hydroxylase and aromatase.

We next turned our attention to embryo transfer in elite racing camels, the necessary prerequisites for which are superovulation (production of many follicles) in the donors and synchronisation of donors with recipient camels. Methods of superovulation involved trying different concentrations of equine chorionic gonadotropin (eCG, Folligon) with or without ovine FSH (Ovugen) or porcine FSH (Follitrophin or Stimufol) and we discovered that most follicles resulted from a combination of 2000 IU eCG and 400 mg of porcine FSH given over a period of four days.

Synchronising the donors and recipients presented more of a challenge as camels do not have the cyclical corpus luteum (CL) of other domestic animals. Therefore, the double prostaglandin injections given 11 days apart, used to synchronise cattle, did not work for camels. We enlisted the help of Professor Greg Adams (University of Saskatoon) and investigated methods using either a combination of oestradiol and progesterone, GnRH and prostaglandin (PG) or follicle ablation and discovered the easiest, and least time- consuming, method was to use a combination of GnRH injected every 15 days with PG injected on Day 7 after each ovulation (Skidmore *et al,* 2009). Not only is synchronisation necessary but the degree of synchronisation required between donor and recipient needed to be defined. Embryos were therefore transferred into recipients that had ovulated from one day before to three days after the donor was mated, and better pregnancy rates were achieved in recipients ovulating 24 – 48 hr after the donor (Skidmore *et al,* 2002). However, it is not always easy to tightly synchronise camels especially if you only have a small number of recipients. Therefore, we investigated methods to prolong the lifespan of the CL using meclofenamic acid, a prostaglandin synthetase inhibitor, so that embryos could be transferred as late as days 8, 10 or 12 after ovulation. By giving 1 g of meclofenamic acid orally from day 7 after ovulation to eight days after embryo transfer, we achieved pregnancy rates of 80%, 60% and 70%, respectively (Skidmore and Billah,

2005). Sometimes, however, follicles in the recipients matured more than 48 hr after the donor was mated, so we explored methods using progesterone injections to supplement the progesterone until the CL could take over production and maintain the pregnancy. We found that by injecting 75 mg of progesterone-in-oil from two days before to three days after embryo transfer we could achieve pregnancy rates between 50 – 60%, compared to 0% in control animals without progesterone supplementation (Skidmore and Billah, 2011). As part of our embryo transfer studies we were also successful in producing identical twins by bisecting an early day 6 blastocyst into two halves using a micromanipulator. Each half embryo was transferring into an individual recipient and Zahi and Bahi were born in 2008 (Fig 4).

After the success of these embryo transfer experiments, interest in embryo transfer grew quickly, not only for producing more of the elite racing or milking camels in the UAE, but also abroad in Iran where attempts were being made to try and preserve the Bactrian camel that was under threat of extinction. It was therefore a pleasure to accept the invitation from Professor Amir Niasari-Naslaji (a long- term friend and colleague) to visit Iran and assist him and his team (Fig 5) in projects to characterise follicular dynamics, multiple ovulation and embryo transfer in the Bactrian camel and then to perform interspecies embryo transfer between dromedary and Bactrian camels. As there were only 150 Bactrian camels in Iran at that time, the idea was to produce multiple embryos from the Bactrian donors and use the dromedary camels as the recipients. The project was successful with the birth of BEHNIA, the first Old World Camelid interspecies embryo transfer, in 2008 (Niasari-Naslaji *et al,* 2009).

In addition to embryo transfer, we have been investigating the use of artificial insemination (AI) in dromedary camels. Elite male racing camels are in high demand for breeding but as their libido and volume of semen produced is low, the use of AI would greatly enhance the number of females that could be covered by a single male. However, camel semen is not easy to work with: firstly, the male has to be trained to the artificial vagina (AV), secondly, the volume is low (usually only 2 – 8 ml per ejaculate) and thirdly, most problematic of all, the semen is very viscous, making mixing it with extender and getting accurate sperm concentration and motility valuations difficult. With the diligent work of my research assistant Mr. Tipu Billah, we trained some of our males to the AV so we could regularly collect semen. Firstly, we investigated several different commercial and home-made extenders but always achieved better results using Green Buffer produced by IMV Technologies (France) specifically for camel semen. Liquefying the semen was the next challenge, and gentle pipetting worked best as, although enzymes such as papain did liquefy the semen, they invariably caused damage to the acrosome or agglutination of the sperm heads. Once we could liquefy the semen we then had to determine the minimum number of sperm to inseminate to obtain pregnancies, where we should inseminate, (either the uterine body or into the tip of the uterine horn) and finally when to inseminate, (at the same time as the GnRH injection given to induce ovulation or 24 hr later, which is closer to the actual time of ovulation). From our studies we concluded that by inseminating 150 million live sperm into the uterine body or tip of the uterine horn, 24 h after injection of GnRH to induce ovulation could achieve a 50% pregnancy rate (Skidmore *et al,* 2006).

The ability to collect semen from bull camels led to another interesting project in trying to hybridise New and Old World camelids, a project instigated by Professor Short, who was fascinated by camelid evolution. The camel family originated in North America around 30 - 40 million years ago. Some camelids subsequently migrated north crossed the Bering Strait into China, Mongolia, the Arabian Peninsula and India and evolved into Old World Camelids (OWC) namely the Bactrian and dromedary camels, whereas others migrated south into South America and evolved into the New World Camelids (NWC) the guanaco, llama, vicuna and alpaca. Whereas all four species of NWC can readily hybridise with one another, and dromedaries and Bactrians can hybridise, NWC and OWC have not hybridised, due to their differences in size and location, even though they have the same diploid chromosome number of 2n =74. With the help of Paul and Sally Taylor, llama ranchers in Montana USA, we acquired some guanacos and llamas and learnt how to handle these somewhat flighty animals. After a few months we were able to collect semen from the male llamas and then inseminated female camels with llama semen and female llamas/guanacos with camel semen. After many attempts we announced the birth of "Rama the Cama" born from a guanaco dam and dromedary camel sire in 1998 (Fig 6) (Skidmore *et al,* 1999). Over the next few years six hybrids were born alive all of which were from llama dam and dromedary camel sire, but interestingly we did not

Fig 1. The Camel Reproduction Centre.

Fig 3. The staff of the CRC.

Fig 5. My collaboration with Prof Amir Nasari-Naslaji and his team in Iran, 2007.

achieve any live births from the reciprocal cross. In terms of size hybrids were closer to the NWC but much stockier. They did not have a hump but did have the longer tail of the camel, the woolly coat of the llama and a footpad halfway between the cloven hooves of the NWCs and the single footpad of the camel. However, they were infertile so the project was not pursued further, but the fact that we have been able to obtain a viable hybrid between a New World and an Old World camelid after 30-40 million years of genetic isolation was truly amazing.

Once we were able to achieve consistently reliable pregnancy results after the transfer of fresh embryos and insemination of fresh semen, it seemed a natural progression to move onto the transfer/insemination of cooled and frozen embryos and semen. There are many advantages to being able to cool and freeze embryos and semen: namely they are much easier to transport nationally or internationally than live animals, meaning that embryo recipients of females to be inseminated do not have to be in the same location as the embryo donors of male camel donate the semen. In addition, if more embryos are recovered than there are available recipients, or recipients are not synchronised with the donors, the embryos can be frozen, stored and only thawed when recipients are ready on their next natural cycle. However, we soon found that cryopreservation of camel embryos and semen had many challenges.

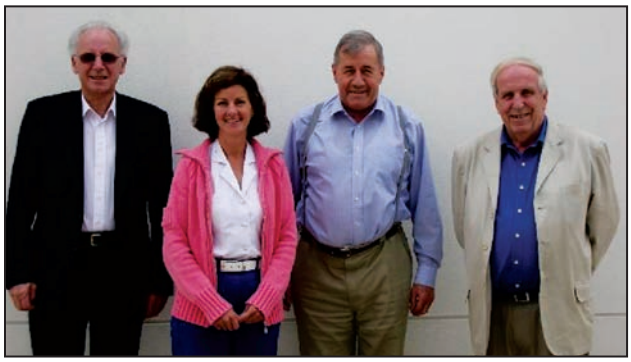

Fig 2. The Governors on the CRC with Dr Lulu Skidmore. (Left to right) Prof. Brian Heap, Dr Lulu Skidmore, Prof Twink Allen, Prof Roger Short.

Fig 4. Zahi and Bahi, genetically identical twins resulting from a bisected early blastocyst.

With the recruitment of more help – that of my good friend and colleague, Dr. Beth Crichton (Omaha Zoo, USA) on a seasonal basis from 2011-2019 and the full-time employment of Dr. Clara Malo in 2013, we made great progress in understanding how to handle and cryopreserve camel semen. Initial experiments investigated methods to improve sperm quality by single-layer centrifugation over a colloid developed by Dr. Jane Morell (Uppsala University, Sweden). This worked well to improve sperm quality and motility but reduced sperm concentration, as inevitably some spermatozoa was trapped in the colloid (Malo *et al,* 2017a). Next, sperm function was evaluated by developing a heterologous IVF system with goat oocytes. As camel oocytes are in

Fig 6. Rama the Cama the world's first camel guanaco hybrid.

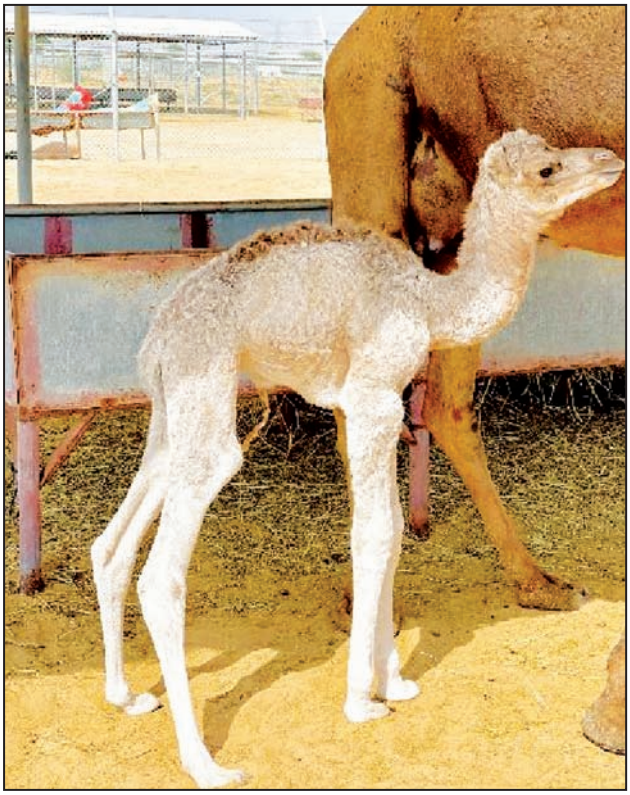

Fig 8. Our first IVF calf born April 2025.

short supply, denuded goat oocytes were incubated with camel sperm to assess the penetration ability of the sperm. Approximately 67% of the sperm successfully penetrated, decondensed and formed pronuclei indicating this is a novel and useful method to evaluate dromedary camel sperm function (Crichton *et al,* 2016). In addition, we investigated the application of cholesterol-loaded cyclodextrins, various cryoprotectants, different freezing and thawing rates and the use of antioxidants to improve post- thaw motility (Crichton *et al,* 2015, Malo *et al,* 2017b, 2018, 2019, 2020). Even though we obtained acceptable post- thaw motilities of around 40 - 45%

Fig 7. The lab staff of CRC. (Left to righ t- front) Dr Murren Herrid, Ali Ahmed, Dr Lulu Skidmore, Dr Clara Malo, Asif Rehman. (back row) Muqtador Billah, Abdel Rahman, Aijaz Hussain.

Fig 9. Participants of the first Short Course in Camel Reproduction held at CRC in 1999.

Fig 10. Lecturing to students.

by cryopreserving camel sperm using 3% glycerol as the cryoprotectant, and employing fast freezing and

thawing rates, pregnancy rates using frozen thawed semen were still disappointingly < 10%. Future research would do well to investigate the appropriate preparation and timing of the recipient for ovulation with respect to insemination of cryopreserved sperm, as synchrony between ovulation and sperm readiness for fertilisation are key to ultimate success.

Cryopreservation of embryos proved to be somewhat easier than cryopreserving semen. In our early studies, in collaboration with Dr. Naida Loskutoff (Omaha Zoo, USA), we were able to cryopreserve embryos by exposing them to 1.5M ethylene glycol for 5 – 10 min and using a slow, controlled rate of freezing (Skidmore *et al,* 2004). However, further studies concentrated on vitrification as it is a faster method that does not require expensive equipment and therefore could be used more easily in the field. In our initial vitrification studies, we achieved similar pregnancy rates to the slow-cooling method of around 37% (Skidmore *et al,* 2005), but this was further improved when Dr. Muren Herrid joined the CRC team in 2014 (Fig 7). After many studies optimising the vitrification method (Herrid *et al,* 2016, 2017), he developed a Camel Vitrification Kit, which we tried and tested at the CRC (Skidmore *et al,* 2020) and it is now commercially available from Minitube (Germany). Although pregnancy rates of 45 – 50 % can be achieved using this method of vitrification, results clearly depend on the quality and size of the embryos, with smaller, good- quality embryos yielding better results (Skidmore *et al,* 2021).

Despite recent advances in cryopreservation of camel embryos, widespread application of this technique is still limited, as transport involving liquid nitrogen is problematic. Therefore, our more recent studies have investigated various cooling and culture methods for short- term preservation of embryos. When my research colleague, Brendan Mulligan, joined us at CRC in 2022, we compared cooling (4°C) and culture (37°C) of varying morphological grade embryos for up to 72 hr. Pregnancy rates of 53.3 and 50% were achieved for good quality, cooled and cultured embryos, respectively after 72 hr. However, although cooling or culture could maintain the viability of good- quality embryos, only embryo culture preserved low morphological grade embryos, as they had poor tolerance to cooling and did not survive 72 hr at 4°C (Mulligan and Skidmore, 2023).

Our lastest achievement was the development of a successful IVF culture system that resulted in the birth of an IVF calf in April 2025 (Fig 8). *In vitro* fertilisation and the production of embryos in camels is difficult due to the lack of a regular supply of oocytes and the challenges involved in collecting good- quality semen, both of which hindered our progress.

Over the years it has been important to us to share the knowledge we have gained from our research, not only in publication of papers in international journals, but also by organising short courses in camel reproduction. The first one was held in 1999 with many national and international participants (Fig 9) and these courses have become so popular now that in 2025 we held two courses (Fig 10).

It has been a great privilege to work with these amazing animals, the "ships of the desert" for the last 33 years and discover some of their reproductive mysteries. The interest in camels is certainly growing worldwide as evidenced by the United Nations declaring 2024 the Year of the Camelid. It was therefore a great honour for me to be invited to speak on our camel reproduction research at a UN meeting in Vienna. I was also invited as a keynote speaker to give lectures about our research at several conferences during "The Year of the Camelid", namely in Morocco, Kuwait, Saudi Arabia (KSA) and United Arab Emirates. Whilst in KSA I visited the Salam Veterinary Group to discuss various possibilities for collaboration between our two laboratories.

I have also acted as a consultant for the Food and Agricultural Organisation (FAO) on a project to protect the Bactrian camel population in Iran. In 2023 we hosted Mr Tavasoli, Mr Veysehzadeh and Mr Markdaneh, (together with Ms Sahereh Joezy as their translator) for a three-week training period at CRC and then in 2024, I advised on the setting up of a mobile laboratory to visit their rural farms and ran a series of online lectures about assisted reproductive techniques in camels. More recently, in June 2025, I was invited by Professor Esengali and his team to visit Kazakhstan to lecture at their summer school held at the Kazakh National Agrarian Research University and to act as a consultant on their camel breeding and genetics project. They were very kind and generous hosts and I thoroughly enjoyed my visit.

With all my recent travels it has been encouraging to see the growing interest in camels worldwide, especially as the many uses of camels and the health benefits of camel milk, for example, become more widely known. I sincerely hope all the work we have carried out at the CRC will continue to inspire people to continue the research and ensure that these amazing animals get the recognition they deserve.

Acknowledgements

I would like to sincerely thank His Highness Shaikh Mohammad bin Rashid Al Maktoum, Vice President and Prime Minister of United Arab Emirates and Ruler of Dubai for his continued support, interest and funding of all our work at the CRC and Dr Ali Redha our Director General. Also particular thanks to the late Prof Roger Short for inspiring me to work with camels and the late Prof Twink Allen for his enthusiasm, encouragement and never ending support throughout my career studying reproduction in horses and camels. In addition, huge thanks to Mr. Tipu Billah and Mr. Aijaz Hussain my research assistant and technician who have been standing alongside me for the last 33 years, to Ali Ahmed, Asif Rehman and all the camel hands who maintain and care for the animals and facilities at the CRC and to Dr. Jane Vaughan (Cria Genesis, Australia), for her invaluable help assisting with veterinary care and embryo transfers during the camel breeding seasons from 2015 – 2020. Also many thanks to Dr. Clara Malo, Dr. Muren Herrid, Dr. Beth Crichton, Dr. Jane Morell, Dr. Budhan Pukazhenthi and Dr. Gregg Adams for their hard work and scientific input that have made the publication of our many scientific papers possible.

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MY JOURNEY TO CAMEL SCIENCE AS A PARASITOLOGIST IN CVRL, DUBAI

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ABSTRACT

This paper reports findings of camel parasites in the United Arab Emirates during a 22-year period between 2002 and 2024. Most of the samples in the first four years were from racing camels in an age group of two to six years while starting from 2006 onwards with the start of operation of a large camel dairy farm, a growing number of samples were from older females and their offsprings. A total of 38 parasite species are mentioned. Some of these parasites were imported with camels from other countries and are exotic for the United Arab Emirates.

Key words: Camel, parasites, United Arab Emirates

My journey to camel science began in 2002 when I started working as parasitologist at the Central Veterinary Research Laboratory (CVRL) in Dubai. At first, I questioned whether this job would be engaging. Since desert winters have only little rainfall, while summer temperatures may rise to 55°C, heating surface of sands goes to nearly 70°C. These are unfavourable conditions for parasites with exogenous development stages.

Parasitology at CVRL was always the smallest department with me as head of the department and varying technical support. Saritha Sivakumar joined the team in 2004, enriching it. Until my retirement in February 2025, our small team worked together to diagnose protozoans, helminths, ectoparasites in many kinds of hosts. The results of parasitological examination were published in annual reports on the website of CVRL (https://www.cvrl.ae). Camel parasite related issues were published in 22 original publications and four book chapters.

During the reporting period, most of the submitted samples were from camels. The number of faecal samples of adult camels processed exceeded 97,000 between 2003 and 2024. During the same period, more than 5,000 camel calves were examined. At beginning, the number of juvenile camels less than one year was small, not more than 25 per annum. This changed with the founding of a big camel dairy, the Emirates Industry for Camel Milk Products (EICMP), that started operation in 2006. Before 2010, most parasitological services for camels were free of charge and the annual number of examined samples reached more than 12,000 in 2009. The introduction of charges sharply reduced this number to less than 4,000 per year. From 2017 onwards, faecal samples from racing camels were processed in the Dubai Camel Hospital and the EICMP farm became the main source for samples that were submitted to CVRL.

We have described our journey to camel parasitology in 6 sections. All new findings in this field were published in various journals of repute and annual reports of CVRL, Dubai. The diverse sections are appropriately cited in the published clinical research by us. We are sure that readers involved in doing research and treating parasitic diseases of Old World camelids will be greatly benefited.

1. Intestinal coccideans

At the beginning, our research focused on camel coccidiosis aiming to develop a vaccine in the future. However, it soon became evident that *Eimeria* infections were not a major issue for UAE dromedaries and that the haemorrhagic enteritis in racing camels was attributed to improper feeding instead of parasitic causes. Three *Eimeria* species (*E. cameli, E. rajasthani* and *E. dromedarii*) were identified with an annual frequency hardly more than 15% (Dubey and Schuster, 2018; 2019). Examination of histological sections of intestines from naturally infected camels led to a detailed description of gamogony of *E. cameli* (Dubey *et al,* 2018). Details of schizogony however, remained unknown. Experimental infection of camels with sporulated *E. cameli* oocysts revealed prepatent and patent periods

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of 31 - 36 and 23 – 43 days, respectively. Despite high OpG values (up to 4,950 per gram faeces) no clinical signs were observed (Gerlach, 2008).

The EICMP farm in Dubai which started operation in 2006 allowed us to study a larger amount of dromedary calves in an age group of less than one year. Thus, additional coccidian species, *Cystoisospora orlovi* and *Cryptosporidium* spp., were detected. *C. orlovi* had been identified in camels from Kazakhstan, India, the UAE, Kenya and recently also from Iraq (Schuster *et al,* 2024). From 2006 to 2024, oocysts of this species were found in 159 of 4,478 dromedary calves. Most infections were fatal, primarily affecting the small colon in calves aged two to seven weeks. Cases of Cystoisosporosis occurred mainly during the calving period from December to April. In adult camels, patent *C. orlovi* infections were diagnosed in just 14 of 77,855 faecal samples (Schuster *et al,* 2017a, Schuster *et al,* 2024). More research is needed to investigate the life cycle of this coccidian species.

Between 2017 and 2024, the carbol-fuchsin test detected *Cryptosporidium* oocysts in 72 out of 972 diarrhoeic camel calf samples. Most of these cases were documented between February and May and involved calves that were less than 60 days old. Due to the difficulty in distinguishing *Cryptosporidium* species by morphology features, molecular tools were used to identify *C. parvum*, *C. meleagridis* and *C. hominis* (Procter *et al,* 2024).

Only 55 of 972 calves and 74 of 886 adult dromedaries from the EICMP farm were diagnosed with *Eimeria* spp. (Schuster *et al,* 2024). These relatively small numbers of a patent infection is a further proof for the low significance of *Eimeria* coccidiosis in camels under desert conditions.

Cystoisospora orlovi and *Eimeria* species are coccidians specific to camels, whereas the detected *Cryptosporidium* species have a broader host range. *C. parvum* commonly infects many ungulate species as well as humans. Birds, such as pigeons or sparrows, may serve as a source for *C. meleagridis*, while humans could be the source for *C. hominis*. Oocysts of *Cryptosporidium* and *C. orlovi* are excreted sporulated, while *Eimeria* oocysts are shed unsporulated. They need moisture and a certain temperature to sporulate. Our experiments showed that camel faecal piles lose almost all moisture within six hours in a hot (at 45 to 55°C ground temperature) and dry desert climate. Already after six hours, these conditions reduced the proportion of oocysts that can sporulate to less than 5 % (Gerlach, 2008).

2. Surra

Surra, caused by *Trypanosoma evansi*, is a significant parasitic disease affecting Old-World camelids. Between 2005 and 2020, 798 (7.4%) out of 10,854 examined camel bold sera at CVRL were tested positive for *T. evansi* antibodies (Schuster *et al,* 2021b). Surra is an arthropod-borne disease with tabanids acting as the primary vectors. Eleven species of tabanids were recorded in the UAE (Ježek *et al,* 2017). These blood sucking insects require damp or swampy breeding grounds. For this reason, primary surra infections in camels occur near oases where horticulture and other plants are intensely irrigated. However, under certain circumstances the stable fly, *Stomoxys calcitrans*, can also play a role as vector. *Trypanosoma evansi* strains in Dubai caused abortions but showed only mild clinical signs in our observations. Oedemas on lower neck, abdomen and distal extremities were often the only clinical signs. Erythrocyte counts and haemoglobin levels were normal, but leucocyte counts often excided $20x10^9$/l. Most *Trypanosoma*-positive camels exhibited lymphocytosis and neutrophilopenia, with platelet counts often below 270×10^9/l. Blood samples with high leucocyte counts and lymphocytosis are suspicious for a surra infection and the buffy coat of these samples should be examined for the presence of trypanosomes (Wernery *et al,* 2020b). Also, serological methods can be applied. The indirect ELISA became positive three weeks after infection and stayed positive for several months after a successful treatment that has eliminated the trypanosomes. It was our experience that recommended therapeutics like melarsomin and quinapyramine, alone or combined, reduced parasite numbers but did not fully eliminate them, indicating potential resistance of the UAE *T. evansi* strain (Schuster *et al,* 2021a; Schuster *et al,* 2022). Further investigation into this matter is essential.

3. Trichomonosis

First cases of trichomonosis in UAE dromedaries were reported by Wernery (1991). The flagellates were diagnosed in 24 out of 48 female camels suffering from endometritis. In preparation of the breeding season, preputial washes from 12 breeding bulls of a camel reproduction farm in Dubai were microscopically examined in August 2018. Quickly moving flagellates were seen in two samples. The analysis of genomic DNA revealed the presence of a species that is closely related to the genus *Tetratrichomonas* and excluded the presence of *Trichomonas foetus*. After flushing the prepuces of the

R.K. Schuster and S. Sivakumar in the parasitological laboratory of CVRL.

R.K. Schuster, P. Nagy and N. Wani on the way to the opening ceremony of the camel conference in Bikaner in 2007.

positive camel bulls with oxidised water, repeatedly investigated samples were found negative after that treatment (Wernery *et al,* 2020a).

4. Gastro-intestinal helminths

Intestinal helminths in UAE dromedaries do not play a major role. *Moniezia expansa* was diagnosed in low prevalence (1.0 -1.6%) mainly in racing camels that were fed with fresh alfalfa. Irrigated alfalfa and grass fields offer good conditions for oribatid mites that act as intermediate hosts for *Moniezia* species. Single cases of *Stilesia vittata, Stilesia globipunctata* and *Avitellina centripunctata* were found only in adult female dromedaries that were imported from abroad (Sudan and Pakistan).

Imported camels from Sudan and Pakistan were also infected with *Haemonchus contortus* and *Haemonchus longestipes* as well as with *Trichostrongylus probolurus* and *Trichostrongylus colubriformis*. These trichostrongylid species require grassland for the

Beside camel parasites the parasitology department of CVRL dealt with parasites of reptiles, cats, falcons, wildlife and many other hosts.

At the camel conference of the Dubai municipality in 2023.

external development of their larval stages; thus, they disappeared over time in a sandy environment. Two camel specific *Nematodirus* species, *Nematodirus dromedarii* and *Nematodirus mauritanicus*, were seen at necropsy of adult camels throughout the whole observation period. Regarding whipworms, two species of the genus *Trichuris, T. globulosa* and *T. skrjabini*, were identified based on nematodes that were found at necropsy. The *Capillaria* species could not be identified since only the typical eggs were seen in coproscopy (Schuster *et al,* 2021b). *Nematodirus* spp., *Trichuris* and *Capillaria* species, complete their larval development within the egg shell and

Excursion to a camel farm in connection with the ISOCARD conference in Almaty in 2015.

The CVRL team carried out a Brucella infection trial.

camels become infected through coprophagy. Two further trichostrongylid nematodes, *Camelostrongylus mentulatus* and *Impalaia tuberculata,* are mentioned in camel literature were found in Dubai so far only in gazelles in a private zoological collection.

Eprinomectin is the only avermectin based active agent that is safe for use in lactating cows without a milk withdrawal period since it is not shed with milk. A treatment trial showed a sharp reduction in egg shedding of trichostrongylid eggs and proved the high efficacy of eprinomectin for the first time also in camels (Schuster *et al,* 2009).

Physocephalus dromedarii, previously regarded as a subspecies of *Physocephalus sexalatus*, is a camel-specific abomasal nematode that has been introduced to the UAE through imported dromedaries. The first case of physocephalosis in Dubai was diagnosed in 2011 (Schuster *et al,* 2013). Necropsy results over four years between 2013 and 2016 indicated that 96 (37.9%) out of 253 adult and 22 (13.8%) out of 159 juvenile dromedaries were infected with *P. dromedarii*. Most infected camels belonged to the EICMP farm, with only seven out of 118 infected with *P. dromedarii* originated from other farms in Dubai. All 68 camels that were sent for necropsy from other emirates

R.K. Schuster and family in the Meguro Parasitological Museum in Tokyo in 2023. Parasites from Dubai dromedaries were deposited in the scientific collection of the museum.

were negative for *P. dromedarii* (Schuster *et al,* 2017b). These data indicated that the parasite was introduced with camels imported from abroad. The scarabeid beetle, *Scarabaeus cristatus*, was identified as main intermediate host. Of 638 beetles collected in two habitats in Dubai, 607 (95.1%) harboured 3rd stage larvae of *P. dromedarii* (Schuster *et al,* 2016a). Smaller scarab beetles of the genus *Aphodius* also hosted larval stages of *P. dromedarii*, while birds, amphibians, reptiles and rodents serve as paratenic hosts (Schuster *et al,* 2015b). After 2016, we noticed a sharp decline in the occurrence of *P. dromedarii*. Only 13 further positive cases were detected between 2017 and 2024. The reason for this is most probably an extended dry period in Dubai without substantial rainfall between March 2016 and December 2017. The long-lasting dry condition had a substantial influence on the population of *S. cristatus* as the main intermediate host. Experimental infections of adult dromedaries with third-stage larvae of *P. dromedarii* indicated that early fourth-stage larvae appeared in the abomasum two weeks post-inoculation and juvenile adults were observed four weeks thereafter. The prepatent period lasted 12 weeks (Schuster *et al,* 2016b).

Parabronema skrjabini is another abomasal nematode with indirect life cycle. The horn fly, *Haematobia irritans*, act as intermediate host. *P. skrjabini* was detected only once in a camel imported from Pakistan.

5. Extra-intestinal helminths

Two types of larval cestodes, hydatids of *Echinococcus* spp. and *Cysticercus tenuicollis*, were identified in dromedaries in the UAE. Most infected animals were imported and in other cases, the owner was unaware about the origin of the

camel. Hydatidosis in indigenous camels is rather uncommon in the UAE for two specific reasons. Dogs as final hosts are mainly kept in cities where they are fed with commercial dog food. Home slaughter of ruminants and camels without proper meat inspection is illegal in the UAE. However, 91 out of 1,630 necropsied adult camels between 2005 and 2020 harboured hydatid cysts. Most of the hydatids were in the lungs while livers were less often affected (Schuster *et al,* 2021b). A molecular examination of hydatids found in camels necropsied at CVRL showed the presence seven different strains of *Echinococcus granulosus* sensu stricto, *E. equinus, E. ortleppi* and *E. canadensis* (Pardinilla, 2024, pers. Communication).

Cysticercus tenuicollis, the larval stage of *Taenia hydatigena* was detected only twice in dromedaries. A three-month-old calf that was imported from Pakistan showed rice grain sized structures under the capsule of the liver and in the liver parenchyma. Most of these structures were already calcified while few others contained bilaterally flatted worms. The identification of these structures as larval stage of *T. hydatigena* was possible only by molecular examination (Schuster *et al,* 2015a). A second case of *C. tenuicollis* was diagnosed in an adult female dromedary from a camel breeding farm. The sender was not aware about the origin of the camel. While smaller cysts in this case were completely calcified, lager pea-sized structures were surrounded by a calcified capsule and contained a jelly substance with an already evaginated scolex. Number and size of rostellar hooks suggested the presence of *C. tenuicollis* which was confirmed by molecular analysis (Schuster *et al,* 2019a). These two cases demonstrated that dromedaries are not typical intermediate hosts of *T. hydatigena* since the larval stages tend to degenerate. Contrary to textbooks, dromedaries do not play a role in the epidemiology of tenuicollis cysticercosis.

In December 2018 a farm in Fujairah on the east coast of the UAE imported pregnant Bactrian camels from west Kazakhstan. An offspring of this herd died at one week old in March 2019. A mixed infection of *Cryptosporidium* sp. and *C. orlovi* was the primary reason of the death. As site finding, three specimens of the filarial nematode *Dipetalonema evansi* (one male and two fertile females) were extracted from the cranial mesentery artery of the dead calf. Blood samples from 20 camels of the imported herd were examined with the modified Knott's test. The test found *Dipetalonema* microfilariae in four of 11 adult camels, including the deceased calf's mother. All nine tested camel calves were negative for microfilariae (Schuster *et al,* 2019b). *D. evansi* is transmitted by *Aedes* mosquitos. The presence of patent adult macrofilariae in a neonatal camel calf is indicative of a congenital infection.

Schistosoma indicum was another extraintestinal helminth that was found in a dromedary in the UAE. The adult female dromedary, imported from Pakistan in March 2020, died in October of the same year. The animal was in poor general condition, with a history of chronic emaciation, inappetence, recumbency and CNS signs. Paratuberculosis was suspected by the sender. The primary pathological changes were observed in both the stomach and the liver. As a site finding, worm-like parasites were found in histological sections of an ileum mesenteric vein. A careful inspection of the remaining mesentery revealed worm-like structures in a vein of the proximal colon. *S. indicum* was diagnosed based on the origin of the host and morphological characteristics of the parasite (Schuster *et al,* 2020).

6. Arthropod infections

Hyalomma dromedarii is a tick that is adapted to hot and dry desert conditions and is the most frequent species found on camels in the region. Camels are the preferred hosts while other desert dwellers like gazelles and oryx were less often found infested. The predilection sites of *H. dromedarii* imagos are under the tail and between the legs of camels while larvae and nymphal stages prefer body parts with longer hair (hump or head). Fully engorged females have a body weight of up to 1.5 g and leave the host at night to find a shelter where they can digest the blood. *H. dromedary* is highly fecund with females producing up to 3,000 eggs. The 'camel' tick does not transmit blood parasites to camels, but it is assumed that a heavy tick infestation can cause tick paralysis (Wernery *et al,* 2024). To treat camels, it is recommended to shear longer hair of the hump and the head. Topical application of flumethrin (Bayticol) is effective against preimaginal stages but does not reach adult ticks engorging in the perianal region and between the legs. Identification and treatment of shelter sites where engorged female ticks hide to digest blood and lay eggs can be very effective to reduce the tick population. Two other representatives of the genus *Hyalomma*, *H. detritum* and *H. anatolicum* were found on cattle and small ruminants in Dubai but not on camels

Mange in Old World camelids caused by the mite *Sarcoptes scabiei* is a highly contagious skin disease that influences the productivity and untreated, it can lead to the death of the camel.

Mange mites are transmitted mainly by direct contact, but items used in the camel practice such as blankets, ropes and halters can also play a role in the transmission. Unrest and pruritus are early clinical signs of mange. Severe cases are characterised by hyperkeratosis and alopecia. The body of the camels with generalised mange is covered with thick crusts and smelly exudate from skin cracks attracts sarcophagid flies. The zoonotic character of *S. scabiei* is another aspect (Hebel *et al,* 2025).

Only few cases of obligate myiasis caused by the nasopharyngeal bot *Cephalopina titillator* were diagnosed at CVRL. The reason for this might be the frequent use of macrocytic lactones (ivermectin and moxidectin) in the camel practice in the UAE. Maggots of the Old-World screwworm, *Chrysomya bezziana*, were found in cutaneous wounds in a group of newly imported camels from Pakistan in May 2017. The animals were still in the quarantine section and were successfully treated with ivermectin and deltamethrin.

In connection with 'foot cancer', several cases of facultative myiasis caused by *Wohlfahrtia nuba* were diagnosed (Tsang *et al,* 2018). Maggots of this fly were also removed from cutaneous wounds of mangy camels.

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JOINT INJECTIONS IN CAMELS: A REVIEW

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ABSTRACT

Joint injections in camels have several practical applications, from lameness localisation and administration of medications, to sampling of synovial fluid. With good technique and anatomical knowledge, the procedure can be effective with low risk of complications. Depending on the location and number of injections to be performed, camels may be injected standing or in recumbency. Preparation of the patient can include washing the animal with a mild-disinfectant shampoo to remove organic matter on the skin surface prior to injecting. Physical restraint methods are combined with low dose xylazine. Full aseptic technique is advised, including preparation of the skin, wearing of sterile gloves and proper aseptic technique. Whilst ultrasound-guidance is preferable for certain joints (e.g. vertebral articular process joints, sacroiliac and hip), others can be injected with good accuracy based on anatomical landmarks and thorough palpation (e.g. shoulder, elbow, radio- and inter-carpal joints, tarsal joints, fetlocks etc). Presence of burn scars and wounds at proposed injection sites can be challenging as needles should not be inserted through inflamed tissue due to risk of iatrogenic joint sepsis. This necessitates good anatomical knowledge and understanding of alternative injection locations. The sensitivity of the dromedary camel to alpha-2 agonists also presents a challenge. In the author's experience, complications associated with joint injections are mainly attributed to anaesthesia-related adverse effects and postinjection reactions to intra-articular medications.

Key words: Arthrocentesis, camel, intra-articular, joint injection

Joint injections in camels have several practical applications: from lameness localsation, administration of medications, sampling of synovial fluid and even injection of contrast for arthrograms. Despite its usefulness, the technique is often under-utilised in camel veterinary medicine. Complications of the procedure can include sepsis, drug reactions ('flares') and injury. However, with good technique and anatomical knowledge, the risk of complications is relatively low. In horses, the technique is widely performed and the risk of iatrogenic septic arthritis is <0.05% when proper aseptic technique is used (McIlwraith *et al,* 2015).

Given the environment that camels are housed in, it is beneficial to consider the working environment prior to embarking on joint injections. Depending on the location and number of injections to be performed, camels may be restrained in recumbency and a sand surface can make aseptic technique challenging. Therefore, ideally, injections are performed in an area with a surface that can be cleaned and disinfected. Other environmental considerations include fly control and a comfortable working temperature. Outdoor air-conditioning units can provide a more comfortable climate, with the added benefit of reducing fly activity due to moving air; however, the wind created can make sterility challenging if items are being blown around.

Preparation of Patient

Depending on the joints to be injected, it is beneficial to wash the camels prior to injections with a mild-disinfectant shampoo. This can help to remove any crusting on the skin surface and clean away any urine or faecal material to reduce the amount of organic matter on the skin surface. It is advisable to wash camels a few hours prior to injection so that the haircoat has dried fully prior to injection.

Although, evidence suggests that clipping the haircoat is not mandatory and doesn't appear to significantly reduce infection risk, provided that a thorough aseptic skin preparation is performed (Graham *et al,* 2007; Lucas *et al,* 2009). However, despite this, it is the author's preference to clip, mainly due to the camel's dense haircoat and the field conditions.

Restraint

Chemical restraint in camels presents a challenge due to their sensitivity to alpha-2 agonists. Achieving an adequate plane of standing sedation can be challenging. For this reason, low doses of

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xylazine are advised (0.1-0.2mg/kg IV) for standing procedures. Additional top-ups can be administered for sedation under recumbency. However, take caution with repeated top-ups under recumbency as it is easy to induce bradycardia, hypotension and respiratory depression with xylazine. Sedation protocols should be combined with physical restraint, including 'lip twitches', leg ties and hobbles.

Technique

Full aseptic technique is advised, including preparation of the skin, usage of sterile gloves and proper aseptic technique. The author's preference is for chlorhexidine scrub by 70% isopropyl alcohol rinse. Contact time is important for aseptic skin preparation, with recommended time ranging from 5-7 minutes for chlorhexidine (Wilson *et al,* 2015). As with all joint injections, use of both radiographic and ultrasonographic guidance may improve accuracy and ensure correct needle placement. Good anatomical knowledge is essential for increased accuracy and to avoid multiple injection attempts which can increase the risk of articular sepsis (Walmsley, 1995; Steel, 2008). Following joint injections, gauze swabs are applied over the injection sites with adhesive bandage dressings which are kept in place for 24 hours to prevent contamination.

Axial Skeleton

Injection of cervical and thoracolumbar articular process joints can be performed using ultrasound-guidance as in horses (Johnson *et al,* 2021; Fuglbjerg *et al,* 2010). Similarly, ultrasound-guided injection of the sacroiliac joint can be performed as in horses, with the cranial approach advised for safety (Stack *et al,* 2016).

Shoulder Joint

The author's preference is a 9 cm, 18G spinal needle. The procedure can be easily performed in the standing animal. Cranial and caudal portions of the lateral humeral tuberosity are easily palpable. The needle is inserted just proximally to this point, between the cranial and caudal portions, in a horizontal plane with the needle parallel to the ground, directed towards the opposite elbow (Moyer *et al,* 2011). The needle is advanced until bone contact is made and then withdrawn slightly. Depth is approximately 5 cm but is largely influenced by limb position. It is not unusual for joint fluid to extend up the spinal needle, particularly in camels in training. Joint fluid can be easily aspirated to confirm correct placement, if necessary. There should be no resistance to injection. Following injection and withdrawal of the needle, aluminium spray is applied over the injection site.

Elbow

The elbow joint can be injected similar to the horse with either lateral or caudal approach and can be injected either standing or in lateral recumbency. The author prefers a caudal approach, using a 9 cm, 18G spinal needle. A notch can be palpated between the caudal aspect of the lateral supracondylar crest and the olecranon of the ulnar. The needle is inserted into the olecranon fossa in a distomedial direction to a depth of approximately 2 inches (Moyer *et al,* 2011). It should be easy to aspirate fluid from the joint and there should be no resistance to injection. Alternatively, the cranial approach can be taken between the lateral humeral condyle and the radial tuberosity, just cranial to the lateral collateral ligament and directing the needle caudally (Alsobayil *et al,* 2015). However, this approach is more likely to induce articular cartilage damage. In the author's experience, camels do not tolerate elbow injections standing as well as the shoulder joint and are more likely to sit down into sternal recumbency. Therefore, the author's preference is to perform this injection in lateral recumbency with the limb in extension. Aluminium spray is applied to the injection site once completed.

Carpus

Joint anatomy of the camel carpus is similar to horses with 3 joints: radiocarpal, intercarpal and carpometacarpal joints. As in horses, the intercarpal and carpometacarpal joints communicate whereas the radiocarpal joint does not communicate (King *et al,* 2022). Radiocarpal and intercarpal joints can be easily accessed through dorsomedial and dorsolateral approaches with the limb in flexion (Moyer *et al,* 2011). The joint should be carefully palpated and the needle inserted either lateral or medial to the extensor carpi radialis tendon. The author preferred a 20G 1.5 inch needle for this procedure. Choice of location is often determined by presence of scarring or wounds due to burning. An alternative approach to the lateropalmar pouch of the radiocarpal joint has been reported with the benefit of avoiding inadvertent puncture of the tendon sheaths, as well damage to the articular cartilage. Puncture site can be palpated in the depression formed by the distal end of the ulna (lateral styloid process) and the accessory carpal bone (King *et al,* 2022). It is the author's subjective impression that the intercarpal/carpometacarpal joint capacity is less than that of the horse.

Fig 1. Clipping of haircoat at injection site for shoulder injection prior to aseptic preparation of scapulohumeral joint.

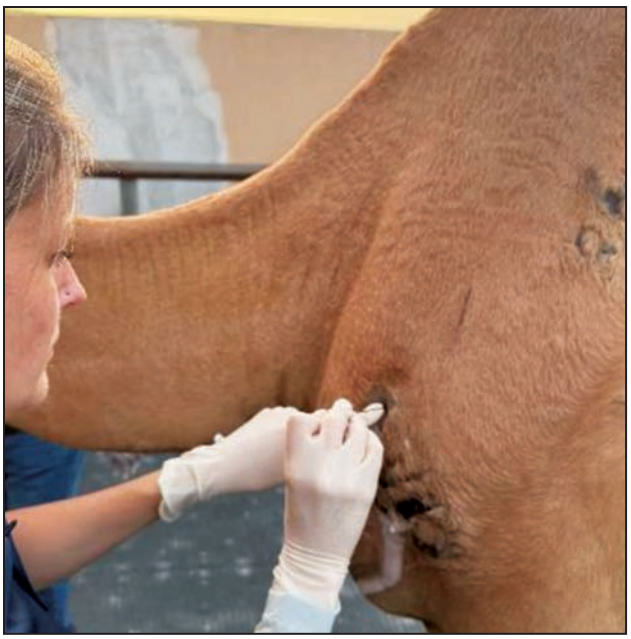

Fig 2. Palpation of anatomical landmarks (cranial and caudal heads of lateral humeral tuberosity) prior to insertion of 18G 9 cm spinal needle for injection of the scapulohumeral joint.

Fetlock

The medial and lateral digits of the fetlock must be injected separately as the joints do not communicate. A dorsal approach is described whereby the joint is flexed and the space for needle insertion is palpated between the distal end of the condyle of the metacarpus and the proximal aspect of P1 (Alsobayil *et al,* 2015). It is the author's preference to inject the dorsal fetlock with the joint in extension, either medial or lateral to the digital extensor tendon. A lateral arthrocentesis approach to the proximal palmar/plantar pouches of the fetlock joints has also been described which has reduced risk of articular cartilage damage as well as avoiding extensor tendons (Al Aiyan *et al,* 2023). The author's preference is a 20G 1.5 inch needle for this injection.

Fig 3. Dorsomedial injection site for tarsocrural joint, distal to medial malleolus of tibia and medial to saphenous vein, using aseptic technique and a 20G 1.5 in needle.

Phalanges

Both dorsal and lateral approaches to the proximal interphalangeal joints have been described. For the distal interphalangeal joint, a dorsal approach must be taken (Alsobayil *et al,* 2015; King *et al,* 2022; Moyer *et al,* 2011).

Hip

A blind approach to the dromedary hip joint has been described by Shawaf *et al* (2023). In this paper, the authors described inserting a needle above the palpable edge of the greater trochanter and directing the needle perpendicular to the vertebral column distomedially. This technique was most easily performed with the camel in lateral recumbency and the limb in flexion. It is the author's opinion that ultrasound-guidance would increase accuracy and the technique is well described in equine literature (David *et al,* 2007).

Stifle

The stifle joint is comprised of the femoropatellar, medial and lateral femorotibial joints. The technique in camels is described by Shawaf *et al* (2023). For femorotibial joints, it is the author's preference to perform using ultrasound-guidance. For this purpose, an 18G, 9cm spinal needle would be used.

Tarsus

The tarsus is comprised of four joints: the tarsocrural (tibiotarsal), proximal intertarsal, distal intertarsal and tarsometatarsal joints. The tarsocrural joint is a large volume, high motion joint. It is easily approached using a 20G 1.5 inch needle, through the dorsomedial pouch, distal to the medial malleolus of the tibia and medial to the saphenous vein (Moyer *et al,* 2011). However, burn scars and wounds may necessitate a dorsolateral approach, lateral to the saphenous vein. If joint effusion is present, plantar pouches (medial and lateral) are easily palpable and can also be utilised. The proximal intertarsal joint may be approached dorsolaterally (distal to the distal end of the calcaneus) or dorsomedially (proximal to the central tarsal bone), whilst the distal intertarsal joint may be accessed dorsomedially (between the central and 4th tarsal bones) (Shawaf *et al,* 2023). In some cases the distal intertarsal joint communicates directly with the tarsometatarsal joint (Shawaf *et al,* 2023). Finally, the tarsometatarsal joint can be accessed through 3 approaches viz. dorsal, caudolateral or caudomedial. Dorsally, it can be approached in the depression between the fused 2nd and 3rd tarsal bones and the 4th tarsal bone. Caudolateral and caudomedial approaches are taken just proximal to the 4th and 2nd metatarsal bones, respectively (Shawaf *et al,* 2023). For these distal tarsal joints in camels, the volume is small and the space is tight. Therefore, it is the author's preference to use smaller gauge needles, such as 23-25G, 1 inch needles. Radiographic guidance is advisable to confirm needle placement in these joints.

Challenges and Complications

Challenges unique to the dromedary camel include difficulties associated with injection sites, burn scars and wounds. Needles should not be inserted through inflamed tissue, as this risks introduction of subcutaneous infection into the joint. This necessitates good anatomical knowledge and understanding of alternative locations for each joint. Additionally, sensitivity of the dromedary camel to alpha-2 agonists presents a challenge which must be navigated. In the author's experience, complications associated with joint injections are mainly attributed to anaesthetic-related risks as well as postinjection reactions to intraarticular medications. Anaesthetic complications encountered include respiratory depression associated with additional top-ups of xylazine in a fractious camel, as well as development of a traumatic semitendinosus haematoma secondary to rolling a camel too vigorously into lateral recumbency. Several joint 'flares' were observed in camels injected intra-articularly with pentosan polysulfate which were managed conservatively. In addition, one case of a severe haemarthrosis was encountered following injection with pentosan polysulfate which responded to needle lavage.

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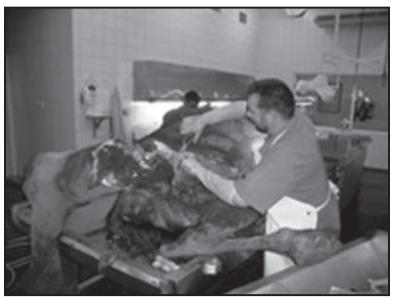
News

LAUDATIO FOR DR. JOERG KINNE AND HIS DISTINGUISHED CAREER IN VETERINARY PATHOLOGY



Dr. Joerg Kinne, CVRL Pathologist, necropsying a camel

The Central Veterinary Research Laboratory (CVRL) in Dubai, United Arab Emirates, announces the recent superannuation of Dr. Joerg Kinne, DVM, *Dr. med. vet.*, following thirty years of exemplary service in veterinary pathology.

Dr. Kinne's career is distinguished by his profound commitment to diagnostic excellence and research. He obtained both his veterinary diploma and his doctoral degree (*Dr. med. vet.*) from the University of Leipzig, Germany. Subsequently, he pursued specialised training in Veterinary Pathology and Tropical Veterinary Medicine. Before relocating to the UAE, Dr. Kinne held academic positions at the Institute of Veterinary Pathology at Leipzig University, where he cultivated extensive experience in diagnostic pathology, research, and disease investigation.

Dr. Kinne joined the CVRL in 1996, where he became a principal veterinary pathologist and a pivotal figure in regional animal health. Over the course of three decades, his extensive experience in diagnostic pathology was applied with particular focus and expertise to camel medicine and pathology.

His contributions have been instrumental in advancing the understanding and control of various pathological conditions affecting the *Camelus dromedarius* (dromedary camel). Through meticulous post-mortem and histopathological analyses, Dr. Kinne contributed significantly to the investigation of infectious and non-infectious diseases crucial to the welfare of racing and breeding camels in the UAE and neighboring countries. This work has been fundamental in supporting the CVRL's role as a leading global centre for camel health and research.

We trust that after thirty years in the lab, he is now enjoying life back in Germany, where no desert sand gets stuck between his books, where the weather will be cold and rainy, and the calls are entirely non-pathological.

From the entire CVRL we thank you, Dr. Kinne, for 30 years of friendship, fun and for almost always having the right answer to tough cases.

GOURMAND AWARD TO JCPR AND DR GAHLOT

The Gourmand award was given to Dr. Tarun Kumar Gahlot, Editor, Journal of Camel Practice and Research in a ceremony in Saudi Feast Food Festival at Riyadh, Saudi Arabia on 30th November 2025. The Gourmand Award is highly relevant to the Journal of Camel Practice and Research (JCPR) because the journal itself was selected for a prestigious category (B27) of the 30th Gourmand Awards in 2024, specifically in the best years of camelid publication category. It was given to Dr. Tarun Kumar Gahlot for his exceptional contribution to veterinary science and camel care. The citation appreciates Dr. Gahlot's innovative skill, vision and commitment to animal welfare and scientific purposes. The JCPR is a blind peer-reviewed international journal that publishes high-quality research and clinical articles on various aspects of camelid practice and research, including camel milk and meat production and nutrition. The Gourmand Awards recognise publications that contribute to global food and drink culture. The JCPR's focus on camel products, such as milk, which is a vital food source for many pastoral communities, directly relates to the Gourmand Awards' interest in diverse and local food cultures. The selection for the award highlights the journal's significant contribution to the knowledge and promotion of camel products as a food source, raising its international profile beyond just the academic veterinary community to include the food and drink sector. In essence, the Gourmand Award acknowledges the cultural and gastronomic importance of the topics the JCPR covers, creating a direct and specific relevance.


GOURMAND
World Cookbook Awards 2025

Best in the World GOURMAND

title Journal of Camel Practice and Research

author Tarun Kumar Gahlot

publisher Camel Publishing House

category Best Year of Camelids Publication

Congratulations, Tarun Kumar Gahlot, on your exceptional contributions to veterinary science and camel care. Your decades of dedication as Professor of Veterinary Surgery and Radiology, along with over 23 years leading the Journal of Camel Practice and Research, have advanced knowledge and practice in the field. Through your innovative surgical techniques, you have demonstrated remarkable skill, vision, and commitment to both animal welfare and scientific progress.

Edouard Cointreau, Chairman of the Awards Committee

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FRACTURE MANAGEMENT IN THE RACING CAMEL

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ABSTRACT

Fractures in racing camels present unique challenges compared to other camelids. The skeletal anatomy of the camel has some distinctive characteristics. Traumatic fractures of most parts of the camel's skeleton have been reported. However, few peer-reviewed articles are available on the fixation of these fractures. The weight, high speed, training methods, training patterns, and racing over set distances at maximal cardiorespiratory exertion on sand have revealed distinct long bone fractures in racing camels. Dorsal metacarpal disease due to repeated stress and microfractures is commonly encountered in young racing camels. Carpal joint osteochondral fragmentation ('chip fractures'), causing lameness in racing camels, similar to those in racehorses, has been identified and treated by arthroscopy. The author's experience in managing and treating fractures in camels at the Dubai Camel Hospital is described.

Key words: Arthroscopy, camels, fractures, management

Camel racing is a centuries-old racing event which has been practiced as a traditional Middle Eastern sport, particularly in the Arabian Peninsula countries of the United Arab Emirates (UAE), Saudi Arabia and Qatar since Medieval times. Camel races hold an important position in Middle Eastern, particularly the Bedouin, culture and society, initially for social events and celebration and now, in the modern multi-million-dollar era, of intense racing competition akin to world flat horse racing. Fractures in racing camels (Arabian camel/dromedary/*Camelus dromedarius*) present unique challenges compared to

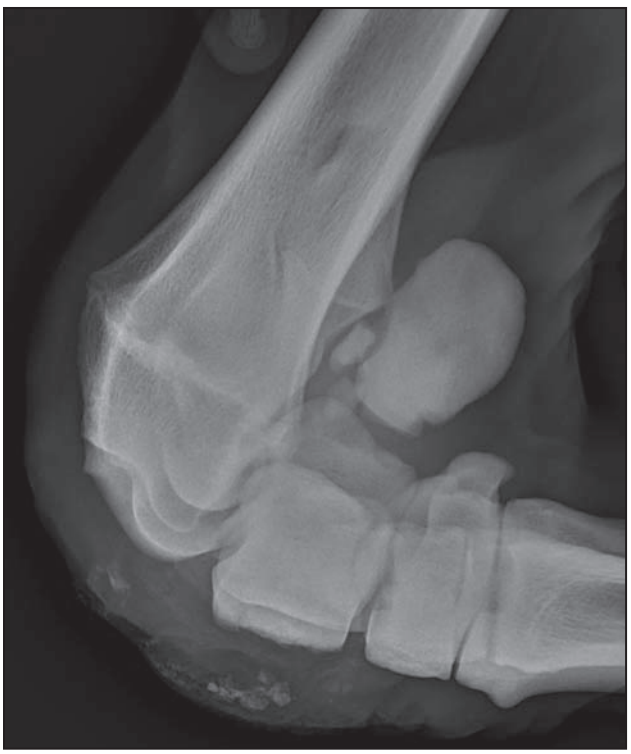

Fig 1. Flexed lateral radiograph of a carpus with a dorsal distal 1/3 comminuted accessory carpal bone fracture in a 2-year-old male racing camel (Courtesy: Dr Morgane Schambourg).

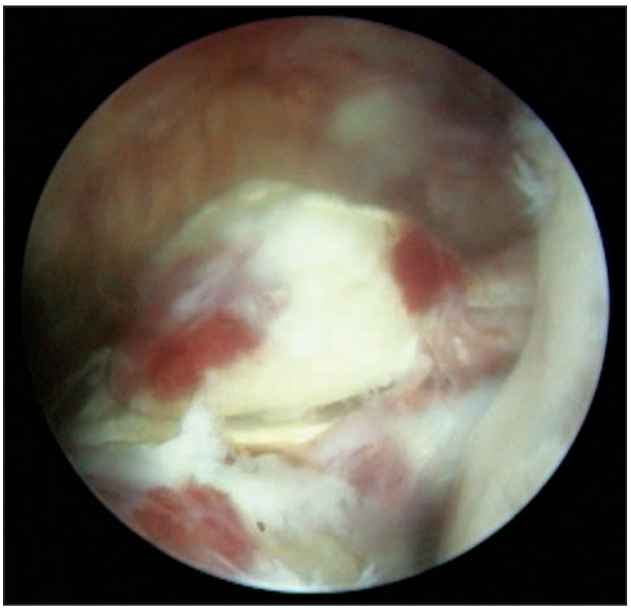

Fig 2. Arthroscopic view of one of the comminuted accessory carpal bone fracture fragments through a palmarolateral radiocarpal joint portal approach from the case in Fig 2 (Courtesy: Dr Morgane Schambourg).

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other camelids let alone compared to other domestic species. The skeletal anatomy of the camel has some distinctive characteristics. New World camelids (llamas, alpacas, guanacos, vicunas) in general are considered excellent patients for the treatment of orthopaedic injuries because of their relatively low body weight, tolerance of external coaptation devices, ability to ambulate on three legs post-operatively and can tolerate prolonged periods of recumbency for recuperation after surgery. The racing camel also shares some of these positive attributes for treating orthopaedic injuries.

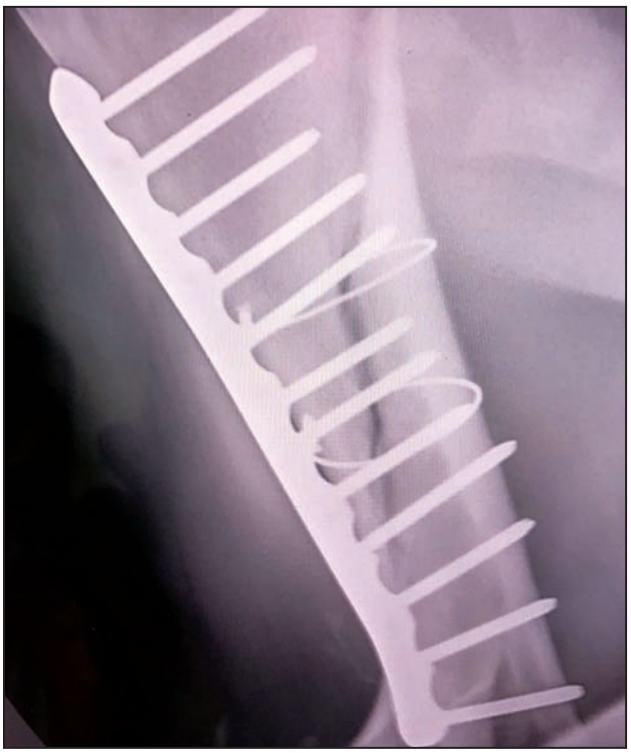

Fig 3. Post-operative mediolateral radiograph of a complete oblique diaphysial humerus fracture fixation using titanium 1.7mm cerclage cables with titanium crimps, a stainless steel 12-hole 4.5/5.0mm locking LCP broad plate and 5.0mm locking screws in a 3-year-old female racing camel.

Traumatic fractures of most parts of the camel's skeleton have been reported. However, few peer reviewed articles on fixation of these fractures are available in the literature except for the mandible. The average weight of a racing camel is far greater than New World camelids with females weighing around 300-540kgs and males 400-690kgs. Intensive breeding programmes including *in-vitro* fertilisation, embryo transfer, cloning and a targeted bloodstock industry for speed have been supported and designed by the Sheikhs over the last 30-40 years. Thoroughbred racing industry rules and regulations (including drug detection), race distances, training methods and nutrition have been adopted and modified for the Arab racing camel. The average speed of the modern racing camel has improved to a point where cantering an average 27-32km/h the first 8km of a 10km race (the most common race distance), increasing to 40-43km/h at the gallop for the next 1 km, then in the final 1 km at the gallop up to 50-55km/h is the norm. The weight, high speed, training methods, training patterns and racing over set distances at maximal cardiorespiratory exertion on sand has revealed long bone fractures similar to those documented in racehorses. Indeed, the concept of stress fractures leading to potential catastrophic fractures of the long bones, in particular the humerus and tibia is raised. Dorsal metacarpal disease (DMD/'sore shins'/'bucked shins') due to repeated stress and microfractures are commonly encountered in young racing camels as they are in young racehorses. Additionally, carpal joint osteochondral fragmentation ('chip fractures') similar to racehorses amenable to arthroscopic management have been identified and treated as a cause of lameness in racing camels.

During 2016-2018, I was in the idiosyncratic position tasked with recruiting expat and local veterinarians and nurses, fully equip a referral hospital, train staff in aspects of modern large animal hospital practice, initiate research and development of camel medicine and make operational a state-of-the-art Dubai Camel Hospital (DCH), a sister hospital to the well-established Dubai Equine Hospital (DEH) at the Al Marmoom racetrack under the patronage of His Royal Highness Sheikh Mohammed bin Rashid Al Maktoum, Vice President of UAE and current Ruler of Dubai.

The hospital was opened by the Sheikh on the 14th December, 2017.

This presentation illustrated some of the challenges we faced as a collaborative team from all corners of the world in the management of various types of fractures encountered, utilising concepts and techniques from large/small animal surgery with the full repertoire of internal and external fixation techniques to choose from and adapted for racing camels.

References can be requested from the author

This presentation was originally given at the European College of Veterinary Surgeons (ECVS) 34th Annual Scientific Meeting, 3-5th July, 2025 in Antwerp, Belgium.

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SEROEPIDEMIOLOGICAL STUDIES FOR THE DETECTION OF ANTIBODIES OF SIX INFECTIOUS DISEASES IN KENYAN DROMEDARY CAMELS

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ABSTRACT

A total of 136 dromedary camel sera were serologically tested for six different infectious diseases to investigate the dromedary camel disease situation in Kenya. The sera originated from different herds and areas of Kenya. Rift Valley Fever antibodies were found in 47% of the camels, followed by *Trypanosoma evansi* with 10.3%, Brucellosis with 7.4%, West Nile Fever with 23.5%, and Blue Tongue with 75.7%. No antibodies were detected for *Peste des Petits Ruminants*. The results found are discussed for every disease.

Key words: Dromedary, Kenya, infections, seroepidemiological studies

Members of the camelid family are robust animals and show a high resilience to many disease agents that cause ailments in domestic animals. A great number of seroepidemiological studies have been performed in camelids, which were summarised by Wernery *et al* (2007 and 2008). However, serological results have a limited predictive value, as they only confirm whether or not the animal has come into contact with an infectious agent and has produced antibodies. The results do not indicate whether the exposure has produced clinically evident disease or how severe the disease response may be. Despite this fact, serological tests are important to help diagnosing and monitoring infectious diseases.

We report here the seroprevalence of 136 dromedary camel sera from Kenya for six different infectious diseases.

Materials and Methods

In April 2025, CVRL received 136 dromedary sera originating from adult camels of both gender from different herds of the Rift Valley in Kenya. They were serologically tested for six infectious diseases. The test methods were as follows:

Rift Valley Fever (RVF)

The RVF inhibition ELISA is a competitive ELISA (c-ELISA) for the detection of antibodies in humans, domestic and wild ruminants. This c-ELISA uses an anti-nucleoprotein horseradish peroxidase (HRP) labelled conjugate which binds to the free nucleoprotein epitopes of RVFV. The conjugate is not directed against the animal species tested and therefore can be used for different animal species, including camelids (Paweska *et al,* 2005).

Typanosomosis (*Trypanosoma evansi*)

The Tryp ELISA used is an indirect in-house ELISA (i-ELISA) which uses a Protein A horse radish peroxidase from *Staphylococcus aureus*. The *T. evansi* parasites were raised in white laboratory rats, and the antigen was prepared according to Rae and Luckins (1984). Anti-camel IgGs are nowadays commercially available and have been produced in rabbits, guinea pigs and laying hens, extracted from egg yolk (Nikbakht Brujeni *et al,* 2009; Wernery *et al*, 2011).

Brucellosis

The brucellosis slide-test is a card agglutination test, also known as Rose Bengal Test (RBT). In the presence of Brucella-specific agglutinins (*B. melitensis, B. abortus, B. suis*), the buffered acidified antigen, stained with Rose Bengal is agglutinated.

West Nile Fever (WNF)

The IDScreen West Nile Indirect is a c-ELISA which detects antibodies directed against the PrME envelope WNV protein. The test uses a conjugate which is directed against the IgG of the WN virus (WNV). The conjugate is not directed against the

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animal species tested, and can therefore be used for different animal species including camelids.

Blue Tongue (BT)

The BT ELISA is a c-ELISA which can be used for any animal species, including camelids. The c-ELISA is designed to detect antibodies directed against the BT virus (BTV) vp7 protein. The conjugate is an anti-vp7-HRP conjugate.

***Peste des Petits Ruminants* (PPR)**

This ELISA has been developed for the detection of serum antibodies to PPR. It is a c-ELISA which uses rabbit anti-nucleoprotein peroxidase labelled polyclonal conjugate. The conjugate is not directed against the animal species tested, and can therefore be used for different animal species, including camelids.

Beside the positive and negative serum controls in each test kits, CVRL has additionally raised antibodies in CVRL dromedary camels using commercially available vaccines or infecting CVRL's dromedaries with the pathogen, as it was done with brucellosis (*B. melitensis*) and *T. evansi*. PPR antibodies could not be raised by artificial infection, as the dromedary camels did not produce any antibodies. These positive sera are also used in CVRL serological investigations and some of them are commercially available.

Results

Table 1 shows the serological results of 136 dromedary camel sera from Kenya tested for six infectious diseases. The chronological order of these six diseases presented in Table 1 follows a special pattern. The pathogens of the first three infectious disorders produce disease in dromedary camels, whereas WNF and BT produce antibodies, but no disease. PPR neither produced antibodies nor infection/disease in dromedary camels.

Discussion

When investigating sera for antibodies in dromedary camels, test kits commercially available or in-house preparations must be evaluated for their suitability, as camelids have a completely different immune system than other mammals. The evaluation studies have been recently performed on 17 different infectious disease agents (Wernery *et al,* 2007, Part I; Wernery *et al,* 2008, Part II). Excellent results are achieved with c-ELISAs, which can be used for any animal species being tested. However, special care should be taken, when direct or indirect ELISAs are in use. These ELISAs often offer anti-ruminant or anti-bovine conjugates and not anti-camel or anti-llama/alpaca-specific conjugates. In general, cross reactivities between anti-species immunoglobulins (IgG) polyclonal antisera exist. It had been shown that dromedary IgG has 74.3% sequence identity to porcine and 73.1% to both equine and bovine and therefore anti-bovine conjugates may be used for ELISAs to detect antibodies against diseases in camelid sera. Additionally, some researchers use with good success horseradish peroxidase-labelled A or G proteins as conjugate, instead. These proteins derived from the staphylococcal cell wall, are highly conserved and bind strongly also with camel IgGs (Wernery *et al,* 2014).

Rift Valley Fever (RVF)

RVF has been present on the African continent since its discovery in Kenya in 1931. It is a significant zoonotic disease which in humans may develop into an uncomplicated influenza–like illness, but can also take the form of haemorrhagic fatal disease. The *Phlebovirus* is transmitted by 23 mosquito species. Strikingly, all of the RVF epizootics described to date have followed unusually heavy rainy seasons, probably indicating a very large insect population as a vector prerequisite (Hübschle,1983). Immense livestock losses were reported during EL NINO in 1990. RVF is an acute to per acute zoonotic disease of domestic ruminants, predominantly in Africa, but globalisation of trade and changing weather patterns is a concern for the spread of RVF out of Africa, which occurred in 2000 to 2001 to Saudi Arabia and Yemen (Shoemaker *et al,* 2002). In recent years, it has become obvious that New World camels (NWCs) and Old World camels (OWCs) can contract RVF (Wernery, 2025; OIE (WOAH), 2018). Serological investigations during these outbreaks revealed high numbers of reactors which were also found with this investigations of nearly 50%. In the Mauritanian outbreak, the RVFV was isolated from diseased dromedary camels, too (EL Mamy *et al,* 2011, 2014). In adult dromedary camels the main clinical signs of RVF is abortion at any stage and young stock may develop a systemic disease. Since 1990 camel diseases were regularly reported from East Africa, mainly Kenya and Ethiopia until today. Some of these outbreaks have been attributed to a *Morbillivirus* similar to PPR (Roger *et al,* 2001; Roger *et al,* 1998; Roger *et al,* 2000) and Pasteurellosis (Bekele, 1999). Newest research, however, has shown that dromedaries are resistant to these two diseases (Wernery *et al,* 2014; Schulz *et al,* 2019). Camel disease

outbreaks in 2024 and 2025 in Kenya and Ethiopia occurred again after heavy rains and clinical signs observed in dromedary camels were similar to RVF as described by EL Mamy *et al* (2011), Khalafalla and Hussein (2021) in Mauritania in 2010. Currently, further investigations, especially virus isolation, are performed on tissue samples from Kenya.

Trypanosoma evansi (Tryps)

The most important protozoal disease of camels is trypanosomosis (named Surra) caused by *Trypanosoma evansi*. The protozoa is mechanically transmitted by blood sucking flies (tabanids) and causes severe losses. Three forms of the disease are known: acute, subacute and the chronic form, which can last months or even years if not treated. The camels become anaemic and anorexic. As the parasite is rarely found in blood in chronic cases, the development of an antibody ELISA was a major breakthrough in the eradication of this disease (Rae and Luckins, 1984). Many different drugs against Tryps are commercially available, but some are resistant due to over use and reduced volume application (Schuster *et al,* 2022).

Brucellosis

Brucellosis in camels is common and has been reported from many different camel rearing countries. It is one of the most severe zoonosis with 500,000 new human cases per year. Brucellosis in breeding camels is found in all known forms, whereby abortion is its most obvious manifestion (Wernery *et al,* 2014). Serological test methods are manifold and have been evaluated for use in dromedary camels (Soellner *et al,* 2018). With 7.4% of reactors in our study, the percentage is in the expected range.

West Nile Fever (WNF)

The West Nile Virus (WNV) was first isolated in the West Nile District of Uganda in 1937 from which it spread all over the world. The disease reached the United States in 1999. WNV can only be transmitted by mosquitoes which become infected when they take a blood meal from a bird carrying the WNV. Horses and humans can be infected by the mosquitoes but they are dead end hosts and can therefore not transmit the virus (Castillo-Olivares and Wood, 2004). The virus can be transmitted to many mammals including camelids and even to reptiles. So far, WNF has only been reported in NWCs as Whitehead *et al* (2006) attributed neurological cases in alpacas and llamas in the US to WNV IgG antibodies in their CSF, but no virus was isolated.

Antibodies against WNV have been detected in sera of dromedary camels in the Middle East, North Africa and Spain. Seroprevalence of Kenyan dromedaries for WNF was 23.5% and 38% in the United Arab Emirates (Wernery *et al,* Part I, 2007) which is a surprisingly high prevalence in an arid country. It is not certain, if the virus produces clinical signs in dromedary camels and therefore, it is important to test any dromedary camel with central nervous signs (CNS) for WNF. The virus, lineage 1a, was recently isolated from a healthy camel calf (Joseph *et al,* 2016).

Blue Tongue (BT)

BT is an acute arthropod-born viral infection of sheep, cattle and wild ruminants with extreme manifestation variability, not only between different ruminant species but also between different breeds of sheep. More than thirty serotypes of the virus have been identified and especially BTV-8 had entered Europe some years ago with severe consequences. Our investigation showed a 75.7% seroprevalence for BT of 136 dromedary camel sera from Kenya. Similar prevalences of BT were reported from NWCs and OWCs and only 2 statements from Fowler (1998) and Henrich *et al* (2007) reported respiratory distress followed by abortion in a llama and alpaca. No disease has been reported in OWCs caused by BT.

Table 1. Serological investigations of antibodies to six diseases in 136 dromedary camel sera from Kenya.

Disease		Results		Test Details	Manufacturer
		Positive	Percentage		
1.	Rift Valley Fever (RVF)	64	47.1	Competitive c-ELISA	ID Vet, France
2.	*Trypanosoma evansi*	14	10.3	In-house direct i-ELISA	Central Veterinary Research Laboratory, UAE
3.	Brucellosis	10	7.4	Rose Bengal Test (RBT)	Linear Chemicals, Spain
4.	West Nile Fever (WNF)	32	23.5	Competitive c-ELISA	ID Vet, France
5.	Blue Tongue (BT)	103	75.7	Competitive c-ELISA	ID Vet, France
6.	*Pestes de Petits Ruminants* (PPR)	0	0	Competitive c-ELISA	BDSL, UK

Peste des Petits Ruminants (PPR)

No PPR-antibodies were found in Kenyan camel sera. Newest research by Schulz *et al* (2019) showed that camelids developed no clinical signs, no viraemia after artificial infection using the Kurdistan/2011PPR strain.

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INSTRUCTIONS TO CONTRIBUTORS

(For the year 2025 to 2027)

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

The Journal of Camel Practice and Research (JCPR) is a triannual journal (April, August and December issues) published in the English language by the Camel Publishing House, 67, Gandhi Nagar West, Near Lalgarh Palace, Bikaner, 334 001 (India). It is in offset print size of 20.5x27.5 cm in two columns with a print area of 17x22 cm. It will be known as **Journal of Camel Practice and Research** with **Volume number** on yearly basis and **Number** on issues per volume basis (in exceptional cases there can be more than three issues in a volume). The editorial policies of JCPR are established by the editor-in-chief and is detailed in this section. Views expressed in papers published in JCPR represent the opinions of the author(s) and do not necessarily reflect the official policy of the author's affiliated institution, or the editor-in-chief.

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