ISSN 0971-6777 (Print)
ISSN 2277-8934 (Online)

JOURNAL OF CAMEL PRACTICE AND RESEARCH

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Volume 25 December 2018 Number 3

In This Issue

JOURNAL OF CAMEL PRACTICE AND RESEARCH

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Bikaner-334001, INDIA

Phone : 0091-151-2527029 (R)
: 0091-151-2521282 (O)
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Cover Design: Fur cutting and camel dance competitions in Bikaner Camel Festival

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Printer: Sankhla Printers, Vinayak Shikhar, Near Polytechnic College, Bikaner-334003, India.
Phone: 0091 - 151 - 2242023

CONTENTS

Volume 25 **December 2018** **Number 3**

NUCLEAR MAGNETIC RESONANCE FOR DETECTING ADULTERATED CAMEL MILK

Journal of Camel Practice and Research, which is now triannual, has completed 25 years of journey and has proven the biggest platform of publication of research related to new and old world camelids. Research on Bactrian camels is getting a good momentum in China. Rapid detection of adulterated camel milk using low-field nuclear magnetic resonance is a new research in this direction. Dr U Wernery and his team at CVRL, Dubai always throw new insight on infectious diseases and accordingly their important contribution in this issue is focused on zoonotic diseases of camelids with special reference to Q-Fever and brucellosis, mastitis caused by *Clostridium septicum* and adhesive pleurisy of lungs caused by *Streptococcus agalactiae*. Important research on differential expression of the coronavirus (MERS-COV) receptor, dipeptidyl peptidase 4, on normal and stimulated leukocytes of dromedary camels was contributed by the scientists of Saudi Arabia. A reproduction scientists team led by Dr Amir Niasari from Iran established protocols for superovulation before ovum pickup. Another reproduction research was contributed by the scientists of Egypt, Saudi Arabia and Sudan, i.e. relationship between the size of the pre-ovulatory follicles, serum concentrations of oestradiol and progesterone, corpus luteum diameter and pregnancy rate, efficiency of single-dose administration therapies for treatment of endometritis and parasitology research on hepatic cystic echinococcosis. This issue contains manuscripts on Alpacas also. Urinalysis of pregnant and non-pregnant alpacas and bactrian camels was contributed by scientists from Czech Republic, while scientists from Auburn University, USA contributed on cyclooxygenase-1 and -2 isoenzyme activity studies in alpaca. Indian scientists contributed research on nutrition, i.e. effect on growth performance of camel calves following azolla (Azolla pinnata) incorporation in pelleted complete feed and production aspect, i.e. financial viability of range land based camel production system and comparison of ghee prepared from camel with other livestock species.

The year 2018 remained important for camel scientists because they got an opportunity to discuss camel research at International Veterinary Congress, Berlin (July) and ISOCARD conference at Morocco (November). Camel scientists would get another opportunity to interact in forthcoming International Veterinary Congress scheduled in May 2019 at London, UK.

Journal of Camel Practice and Research published 46 papers in the year 2018 out of which 35 were of dromedary research, 8 manuscripts of Bactrian research and 3 were related to New World Camelids. These issues of volume 25 were equivalent to a mega-book and comprised of 326 pages. This volume not only contained manuscripts based on all aspects of camel science but also contained review papers and book review. I am sure that year 2019 will provide more research literature and clues to the future camel research. Merry Christmas and Happy New Year 2019 to all the authors and members of the editorial board of JCPR.

(Dr. T.K. Gahlot)
Editor

DOI : 10.5958/2277-8934.2018.00033.4 Vol 25 No 3, p 249-253

DIFFERENTIAL EXPRESSION OF THE CORONAVIRUS (MERS-COV) RECEPTOR, DIPEPTIDYL PEPTIDASE 4, ON NORMAL AND STIMULATED LEUKOCYTES OF DROMEDARY CAMELS

Abdullah I.A. Al-Mubarak

Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, Al Ahsa, Saudi Arabia

ABSTRACT

The present current study was dune to evaluate the expression pattern of DPP4 on unstimulated and lipopolysaccharide (LPS)-stimulated peripheral blood leukocytes of dromedary camel using flow cytometry. Unstimulated camel blood monocytes showed the highest expression of DDP4 in comparison to unstimulated lymphocytes and granulocytes. The mean fluorescence intensity of DPP4 on unstimulated lymphocytes was significantly higher than that on unstimulated granulocytes. *In vitro* stimulation of whole blood with LPS induced a selective upregulation of DPP4 on monocytes. Together, these results imply that camel monocytes may play a central role in pathogenesis or immune response to MERS-CoV.

Key words: Camel, coronavirus, dipeptidyl peptidase 4, leukocytes, middle east respiratory syndrome (mers-cov)

Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging zoonotic pathogen which has spread to more than 25 countries in the world causing a severe respiratory disease in human with high mortality rate (Zaki *et al,* 2012; Inn *et al,* 2018). According to recent studies, dromedary camels may play a central role in virus transmission to humans (Reusken *et al,* 2013; Adney *et al,* 2014; Reusken *et al,* 2014a; Reusken *et al,* 2014b; Gossner *et al,* 2016; Haagmans *et al,* 2016; Harcourt *et al,* 2018).

Dipeptidyl peptidase 4 (DPP4), also known as CD26, is a type II transmembrane glycoprotein involved in the cleavage of dipeptides and degradation of incretins (Lambeir *et al,* 2003). DPP4 has been recently identified as a functional receptor for the MERS-CoV (Ohnuma *et al,* 2013; Raj *et al,* 2013; van Doremalen *et al,* 2014). MERS-CoV uses its spike protein S1 to bind on DPP4 before entering the cell (van Doremalen *et al,* 2014; Widagdo *et al,* 2016). The expression level of CD26 on the surface of cells of respiratory tract has been found to be correlated positively with susceptibility to MERS-CoV infection (Cai *et al,* 2014; Meyerholz *et al,* 2016). CD26 monoclonal antibody was also able to inhibit MERS-CoV infection *in vitro* (Ohnuma *et al,* 2013).

In human, DPP4 is widely expressed in different tissues including cells of the immune system (Pierson *et al,* 2008). Under human blood leukocytes, DPP4 was mainly found on human T lymphocytes rather than monocytes. For the dromedary camel, the differential expression of DPP4 on blood leukocytes is currently not available. Further the impact of bacterial infections on the expression of DPP4 on camel leukocytes has not been studied yet.

The objective of the present study was therefore to evaluate the expression pattern of DPP4 on unstimulated and lipopolysaccharide (LPS)-stimulated peripheral blood leukocytes of dromedary camel.

Materials and Methods

Animals and blood sampling

Blood samples were collected from 9 apparently healthy dromedary camels (*Camelus dromedarius*) aged between 2 and 4 years at Camel Research Centre, King Faisal University, Al-Ahsa, Saudi Arabia. Blood was obtained by venipuncture of the vena jugularis externa into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany).

Whole blood stimulation

Blood from healthy camels (n=9 animals) was incubated without or with Lipopolysacharide (LPS; 1 µg/ml; Sigma-Aldrich, Germany) for 4 h (37°C, 5%

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CO_2). After incubation, the blood was diluted with phosphate buffer saline (1:1) and centrifuged at 4°C for 10 min at 1000xg. After removing the supernatant, the cell pellet was resuspended in PBS.

Hypotonic lysis of erythrocytes and leukocytes separation

Separation of whole camel leukocytes was performed after hypotonic lysis of blood erythrocytes (Hussen *et al,* 2017). The blood was suspended in distilled water for 20 sec and double concentrated PBS was added to restore tonicity. This was repeated (usually twice) until complete erythrolysis. Separated cells were finally suspended in MIF buffer (PBS containing bovine serum albumin (5 g/L) and NaN_3 (0.1 g/L)) at 5 x 10^6 cells/ml. Cell purity of separated leukocytes was assessed by flow cytometry according to their FCS/SSC properties and always exceeded 90 %. The mean viability of separated cells was evaluated by dye exclusion (propidium iodide; 2 µg/ml, Calbiochem, Germany) and it was above 90%.

Immunofluorescence and flow cytometry

Separated camel blood leukocytes ($4x10^5$) were incubated with monoclonal goat IgG antibody (R and D Systems) specific for CD26 molecules (Pierson *et al,* 2008; van Doremalen *et al,* 2014) in PBS containing bovine serum albumin (5 g/l) and NaN_3 (0.1 g/l). After 30 minutes incubation (4°C), cells were washed twice and incubated with a Alexa Fluor 488a-labelled rabbit F(ab')2-anti-goat IgG (H+L) secondary antibody. After incubation (30 minutes; 4°C), labelled cells were washed twice and analysed on the flow cytometer. A Becton Dickinson FACS Calibur equipped with Cell Quest software (FACS Calibur; Becton Dickinson Biosciences, San Jose, California, USA) was used to collect the data. At least 100,000 cells were collected and analysed with the flow cytometric software FCS Express software Version 3 (De Novo Software, Thornton, Ontario).

Statistical analyses was performed with Prism (GraphPad) and the results were presented as means ± S.E. values. Differences between means were tested with one-factorial analysis of variance (ANOVA) and Bonferroni's correction for normally distributed data. Results were considered significant at a p-value of less than 0.05.

Results and Discussion

The present study analysed the differential expression of dipeptidyl peptidase 4 (DPP4), the receptor for the Middle East respiratory syndrome coronavirus (MERS-CoV), on the main populations of peripheral blood leukocytes of dromedary camels using flow cytometry. For the detection of camel DPP4, a goat polyclonal antibody to human DPP4, which has shown cross-reactivity with camel DPP4 (Widagdo *et al,* 2016), was used to label separated camel leukocytes. As shown in Fig 1, unstimulated camel blood monocytes showed the highest expression of DDP4 in comparison to unstimulated lymphocytes and granulocytes. The mean fluorescence intensity of DPP4 on unstimulated lymphocytes was significantly higher than that on unstimulated granulocytes. On the contrarily, DPP4 has been found to be mainly expressed on human blood lymphocytes (Ibegbu *et al,* 2009), while human blood monocytes showed only a weak expression of DPP4.

To investigate the effect of stimulation with a bacterial pathogen-associated molecular pattern (PAMP) on the expression level of DPP4 on leukocyte populations, camel whole blood was stimulated with Lipopolysaccharide, a cell wall component of gram-negative bacteria and DPP4 expression was comparatively analysed in stimulated and unstimulated blood leukocytes (Fig 2). While the expression level of DPP4 on lymphocytes and granulocytes did not differ between stimulated and unstimulated cells, LPS-stimulated monocytes increased their DPP4 expression significantly (Fig 2).

This seems to be also in line with observation in human patients with chronic lung diseases, such as COPD, where DPP4 expression was increased on activated alveolar macrophages near sites of disease (Meyerholz *et al,* 2016). In addition, human monocytes increased their DPP4 expression upon activation (Zhong *et al,* 2013). Differential expression of CD26 in the upper respiratory tracts has been shown to be responsible for higher susceptibility of humans for MERS-CoV infection than dromedary camels (Widagdo *et al,* 2016). The results of the current study suggest that preexisting bacterial infections could increase MERS-CoV receptor expression and therefore increase animal susceptibility to MERS infection. However, more studies are needed to evaluate the impact of bacterial infections on DPP4 expression on epithelial cells of respiratory tract.

The results of the current study showed different expression pattern of DPP4 on camel and human blood leukocytes and agreed with the observations regarding the increase of DPP4 expression on activated human monocytes. These results may contribute to better understanding of disease pathogenesis and immune response toward MERS-CoV in camel.

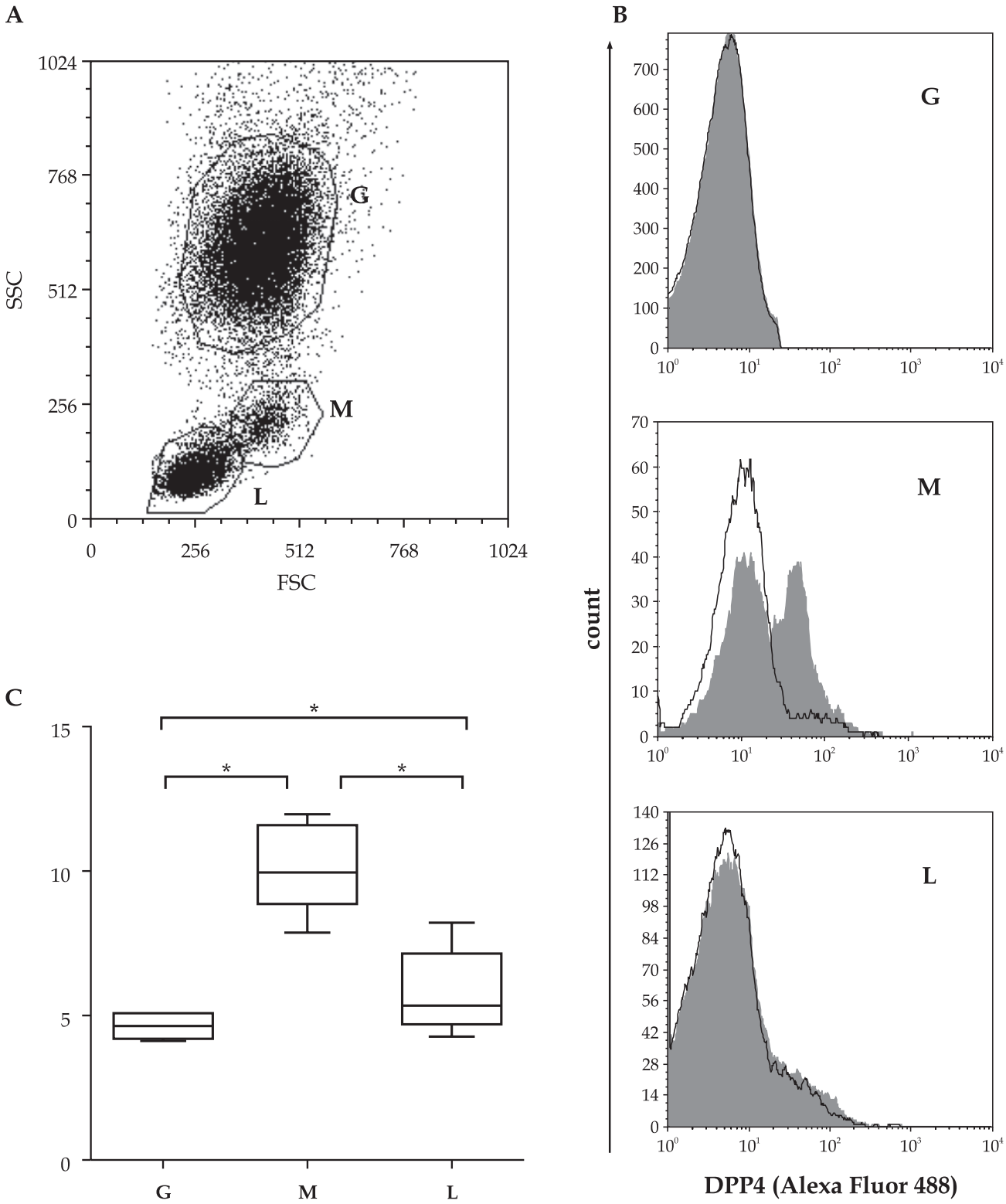

Fig 1. Gating strategy and expression pattern of DPP4 on main leukocyte populations in peripheral blood of dromedary camel. Camel whole leukocytes were separated by hypotonic lysis of erythrocytes and labeled with polyclonal antibody to DPP4 (CD26) or with isotype control and analysed by flow cytometry. A) In a SSC/FSC dot plot, camel granulocytes (G), monocytes (M) and lymphocytes (L) were defined according to their forward and sideward scatter characteristics. B) After setting a gate on granulocytes, monocytes and lymphocytes, the mean fluorescence intensity (MFI) of DPP4 (gray filled histogram) or isotype control (black lined histogram) was presented in overlay histograms. C) MFI of DPP4 expression on granulocytes (G), monocytes (M) and lymphocytes (L) was calculated and presented graphically as mean ± SEM (* = $p<0.05$).

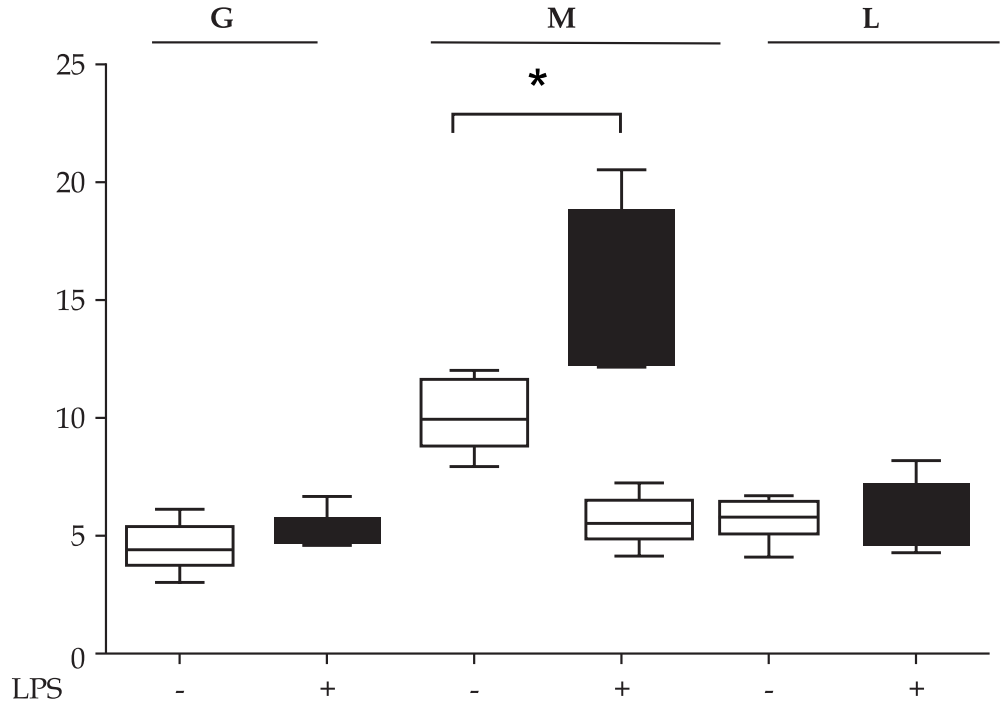

Fig 2. Impact of whole blood stimulation with LPS on the expression level of DPP4 on main leukocyte populations in peripheral blood of dromedary camel. Camel whole blood was stimulated for 4h with LPS and separated leukocytes were labeled with polyclonal antibody to DPP4 (CD26) or with isotype control and analysed by flow cytometry. After setting a gate on granulocytes, monocytes and lymphocytes, the mean fluorescence intensity (MFI) of DPP4 expression on granulocytes (G), monocytes (M) and lymphocytes (L) for stimulated and unstimulated leukocytes was calculated and presented graphically as mean ± SEM (* = $p<0.05$).

Acknowledgement

The author thanks the Deanship of Scientific Research, King Faisal University, Saudi Arabia for the financial support of this work (grant number: 150228). The author is also thankful to the Research Centre of Camels, King Faisal University, Saudi Arabia for providing blood samples.

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DOI : 10.5958/2277-8934.2018.00034.6 Vol 25 No 3, p 255-260

DETERMINATION OF WHOLE BLOOD LEVELS OF CYCLOOXYGENASE-1 AND -2 ISOENZYME ACTIVITY IN ALPACA (*Vicugna pacos*)

J.J. Kottwitz, M.A. Edmondson[1] and D.M. Boothe
Department of Anatomy, Physiology and Pharmacology
The Department of Clinical Sciences[1], College of Veterinary Medicine, Auburn University, AL, 36849, USA

ABSTRACT

This study determined concentrations of thromboxane B_2 (TxB_2) and prostaglandin E_2 (PGE_2) and metabolites (PGEM) in alpacas utilising commercially available assays. Twenty healthy adult alpaca (11 castrated males and 9 females) participated in this study. Four ELISAs were utilised: two to quantitate TxB_2 in serum as an indicator of *in vitro* cyclooxygenase-1 (COX-1) activity, one quantitated PGE_2 and a fourth assay quantitated prostaglandin E_2 and metabolites (PGEM) in plasma as indicators of *in vitro* cyclooxygenase-2 (COX-2) isoenzyme activity after leukocyte exposure to lipopolysaccharide. Known standards were utilised for confirmation of assay results. Alpaca serum TxB_2 concentrations were substantially lower than reported in other species and those quantitated in four clinically normal horses using identical methods (alpaca=616±294, 95% CI [47, 753] pg/ml; horse=6087± 855, 95% CI [3964, 8210] pg/ml). Alpaca plasma mean PGEM concentration was 50±28, 95% CI [21, 111] pg/ml. Whole blood determination of TxB_2 and PGE_2 have been utilised in multiple species to evaluate COX isoenzymes for evaluating nonsteroidal anti-inflammatory drug (NSAID) COX inhibition. This study demonstrates that TxB_2 as measured using these methods may not be an acceptable method for determination of COX-1 response to NSAIDs in alpaca. The low whole blood TxB_2 levels identified by these assays may also indicate an intrinsic sensitivity to these drugs that warrants further investigation.

Key words: Alpaca, COX-1, COX-2, cyclooxygenase, prostaglandin E_2, thromboxane

Cyclooxygenase-1 (COX-1) is constitutively produced as it is required for essential physiological functions (Kujubu *et al,* 1991; Morita *et al,* 1995; O'Banion *et al,* 1991; Papich, 2008; Ren *et al,* 1995; Xie *et al,* 1991). The prostanoids produced by COX-1 may act as local signaling mediators and hormones mitigating control of intrinsic protective mechanisms of renal, gastrointestinal, or platelet function (Kawahara *et al,* 2015; Smith *et al,* 1994; Vane *et al,* 1998; Wilson *et al,* 2004). COX-2 is generally thought to be induced in response to injury, trauma and other inflammatory stimuli, with associations with the control of cellular division, angiogenesis and cardiovascular functions, as well as a role in influencing water and electrolyte balance controlled by the kidneys (Kawahara *et al,* 2015; Morita *et al,* 1995; Ren *et al,* 1995; Streicher and Wang, 2008; Timmers *et al,* 2007; Vane *et al,* 1998; Wilson *et al,* 2004). COX-1 is thought to play a role in tissue blood flow during inflammatory states, while the uncontrolled action of COX-2 may initiate the undesirable effects of the inflammatory process (Duz *et al,* 2015; Vane *et al,* 1998). COX-2 is considered more efficient than COX-1 in oxygenating eicosapentaenoic, gamma-linolenic, alpha-linolenic and linolenic acids, the key fatty acids involved in the inflammatory cascade (Kawahara *et al,* 2015; Otto and Smith, 1995). Because the function of both COX-1 and 2 are physiologically important, understanding the impact of therapeutic drugs on their function influences predictions of drug safety and efficacy.

Accordingly, understanding species differences in the expression of these two isoforms might facilitate predicting safety and efficacy of cyclooxygenase inhibitors. Many of the adversities associated with NSAID administration may be considered the sequelae of COX-1 inhibition (Amagase *et al,* 2014; Rodrigues *et al,* 2010; Sinha *et al,* 2013; Jones and Budsberg *et al,* 2000). Drugs that target COX-2 preferentially may be regarded as safer; however, species differences in enzyme catalytic activity could mitigate the safety of these drugs. The purpose of this study was to determine concentrations of TxB_2 as an indicator of COX-1 isoenzyme activity and concentrations of PEGM as an indicator of the

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inducibility of COX-2 isoenzymes in alpacas utilising commonly utilised, commercially available assays as a prelude to investigating response to drugs that may alter cyclooxygenase isoenzyme levels.

Materials and Methods

Two commercially available ELISA kits were used to determine Thromboxane (TxB_2) levels in serum as an indicator of COX-1 activity: Assay 1a (Thromboxane B_2 Express EIA) followed by Assay 1b (Thromboxane B_2 EIA, Cayman Chemical Co., Ann Arbour, Michigan, USA). Prostaglandin E_2 (PGE_2) was measured in plasma as an indicator of COX-2 activity using 2 commercial ELISA kits: Assay 2a (Prostaglandin E_2 Express EIA kit) and Assay 2b (Prostaglandin E_2 and Metabolites EIA, Cayman Chemical Co., Ann Arbor, Michigan, USA).

This study was approved by the Institutional Animal Care and Use Committee of Auburn University, Alabama (Protocols: 2013-2039 and 2014-2549). All animals in this study underwent a minimum 6 month washout period prior to sample collection. Blood for pilot data analysis was obtained from 7 adult alpacas (4 castrated males and 3 intact females) randomly selected from the Auburn University Small Ruminant Herd. Because TxB_2 was undetectable using Assay 1a, the full study using blood collected from a total of 20 randomly selected alpacas (11 castrated males and 9 intact females) was analysed using only Assay 1b. All animals were housed in an outdoor paddock on the veterinary campus.Three adult male castrated horses (two Thoroughbreds and one American Quarter Horse) and one adult female Percheron, privately owned by one of the authors, served as positive controls. These animals were maintained solely on free range costal Bermuda grass pasture. Blood was collected on premises from these animals.

Collection and analysis of both alpaca and horse blood utilised the same methodology. Blood (10 ml) was collected aseptically from the jugular vein, using a 12 ml syringe and an 18 gauge, 1.5 inch (38 mm) needle. All samples were processed and analysed in triplicate immediately after collection with processed serum and plasma stored at -20°C for repeat analysis. All assays (Assay 1a, 1b, 2a, 2b) were repeated to verify results.

Determination of TxB_2 as an indicator of COX-1, was performed by collecting 10ml of blood and immediately transferring the blood to a plain glass blood collection tube (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, New Jersey 07417, USA). Blood was incubated at 20°C for 2 hours, then centrifuged twice at 2000 x g for 10 minutes (Beretta *et al,* 2005). The supernatant serum was harvested and divided into 250 µl aliquots prior to analysis. Pilot serum samples were analysed with Assay 1a according to the manufacturer's instructions, using the purification option to remove contaminants that may interfere with the assay. This methodology provided a manufacturer's specified detection limit of 45 pg/ml for undiluted samples. Samples were purified with ethanol to precipitate proteins and filtered through a disposable extraction column (Bakerbond SPE Otadecyl, C-18 extraction columns, Mallinckrodt Baker, Inc, Phillipsburg, New Jersey 08865, USA). For Assay 1b, serum samples from the group of 20 alpaca were processed utilising the same methodology as above, but without the purification step as per the manufacturer's instructions for that specific assay. This methodology provided a manufacturer's specified detection limit of 5 pg/ml for undiluted samples. Samples for both Assay 1a and 1b were diluted 1:10, 1:25, 1:50 and 1:100 with EIA buffer after purification. Known dilutions of assay standard were aliquoted for measurement with each plate for quality control purposes.

For determination of PGE_2, 10 ml of blood was collected as for COX-1 (via TxB_2) determination, then immediately transferred to a 10 ml lithium heparin blood collection tube (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, New Jersey 07417, USA) to which 50 µg *E. coli* LPS, serotype 026:B6 (Sigma Aldrich Co., 3050 Spruce Street, St Louis, Missouri 63103) to induce COX-2 and 100 µg acetylsalicylic acid to inhibit COX-1 (Beretta *et al,* 2005) were added. Samples were transferred to 10 ml glass tubes, incubated at 37°C for 24 hours, then centrifuged twice at 2000 × g. Plasma was harvested, re-centrifuged at 2000 × g, then divided into 250 µl aliquots (Beretta *et al,* 2005). Samples analysed with Assay 2a were purified per the manufacturer's instructions which was as with Assay 1a to remove possible contaminants. Samples analysed with Assay 2b were purified by acetone precipitation for removal of proteins. This difference in purification allowed for the detection of PGEM. This methodology provided a detection limit of 36 pg/ml for Assay 2a and 2 pg/ml for Assay 2b. The supernatant was removed and dried under nitrogen. Purified samples for each PGE_2 assay were diluted at 1:10, 1:25, 1:50 and 1:100 with commercially available buffer (PGE EIA Assay Buffer, Cayman Chemical Co., Ann Arbor, Michigan, USA).

The standard curves for each assay were derived from the manufacturer's standard provided with

each assay kit, prepared per the manufacturer's specifications. The concentration of TxB_2 or PGE_2 and metabolites in each sample were calculated from the standard curve for each dilution per the manufacturer's instructions and as described in previously published studies (Beretta *et al,* 2005; Brideau *et al,* 2001). All assay plates were read at a wavelength of 415 nm and all results are reported as mean ± 1 standard deviation as calculated using commercially available software (Microsoft Excel, Microsoft Redmond campus, Redmond, Washington, USA).

Results

Results of all assays performed are summarised in Table 1. Thromboxane concentrations were not detectable within the linear range of the standard curve in alpaca serum at any dilution utilising assay 1a in the serum of the 7 pilot alpacas, whereas equine serum TxB_2 concentrations were 4146 ± 229 pg/ml, 95% CI [3829, 4463].

The Thromboxane Express (TxB_2 Express) assay did not show measurable results in Alpaca. The Thromboxane Assay (TxB_2) assay showed measurable results for alpaca, but those results were 1/10 of the results for horses utilising the same assay.These results indicated a substantially lower level of Thromboxane activity as an indicator of intrinsic Cycloxygenase-1 activity in alpacas compared to horses. This low COX-1 level will complicate thorough evaluation of drugs affecting COX-1 levels and may be a predisposing factor for well documented health problems in alpaca, such as C3 ulceration. Prostaglandin E_2 and metabolites levels were comparable between horses and alpaca, suggesting that inducibility of COX-2 is comparable between these two species.

Serum TxB_2 concentrations in the full study based on Assay 1b were 616 ± 294, 95% CI [478, 753] pg/ml at the 1:10 dilution. All other dilutions were below the detection limits of the assay.Equine mean serum TxB_2 levels were 6087 ± 855, 95% CI [3964, 8210] pg/ml at the 1:10 dilution using the same methodology.

PGE_2 was detectable in both horses and alpaca with assay 2a. In alpaca, the mean concentration was 34 ± 10, 95% CI [27, 41] pg/ml and for horses, mean concentrations were 34 ± 6, 95% CI [25, 43] pg/ml. For PGEM, concentrations were detectable in both horses and alpaca at the 1:10 dilution with assay 2a. In alpaca, the mean concentration was 50 ± 28, 95% CI [21, 111] pg/ml and for horses, mean concentrations were 34 ± 6, 95% CI [26, 43] pg/ml.

Discussion

The results determined in this study indicate that these commercially available ELISA kits are not appropriate bioanalytical assays to evaluate COX activity in alpaca. Evaluation of COX activity as a means of *in vitro* evaluation of NSAID drugs was first published in human medical literature (Patrignani *et al,* 1994). It is important to distinguish between the presence of COX isoenzymes and the activity of these enzymes. The specific inhibition of the activity of these isoenzymes is the target of NSAID drug therapy. Because of this, quantification of enzyme activity is an important component of understanding how NSAIDs function in different animals and describing how different drugs target both COX-1 and COX-2. The original human study utilised a combination of radioimmunoassay and Western blot to determine whole blood PGE_2 and TxB_2 concentrations via radioimmunoassay detection of H3 labeled [3H] PGE_2 or [3H] TxB_2 and specific rabbit anti-PGE_2 or anti-TxB_2 sera (Patrignani *et al,* 1994). Thromboxane A2 (TxA2) is produced from arachidonic acid metabolism via COX-1 activity by

Table 1. Summary of Assays performed at a 1:10 dilution for both Alpaca and Horse.

Dilution 1:10							
Assay	**Name of assay**	**Species**	**Number of Animals**	**Mean (pg/ml)**	**Standard Deviation**	**Low Range (pg/ml)**	**High Range (pg/ml)**
1a	TxB_2 Express	Alpaca	7	not detectable	---	---	---
1a	TXB_2 Express	Horse	4	4146	± 229	3984	4308
1b	TxB_2	Alpaca	20	616	± 294	329	1487
1b	TxB_2	Horse	4	6087	± 855	5483	6692
2a	PGE_2 Express	Alpaca	7	34	±10	27	41
2a	PGE_2 Express	Horse	4	34	± 6	25	43
2b	PGE_2 + metabolites	Alpaca	20	50	± 28	21	111
2b	PGE_2 + metabolites	Horse	4	34	± 6	30	39

multiple cells within the body, but is considered to not be a measurable circulating hormone as it is rapidly hydrolysed to TxB_2, which is then metabolised slower and eventually eliminated from the body by the kidneys (Patrignani *et al,* 1994; Patrono *et al,* 1986).

Previous studies have demonstrated variability in intrinsic TxB_2 levels in domestic animals, with horses, pigs and cats having much lower concentrations than dogs or cattle (Patrono *et al,* 1986; Reinke and Brune, 1988 and Van Hoogmoed *et al,* 2004). Horses were utilised as a comparative control for this study because of previously published studies having documented this intrinsically lower level. The mean TxB_2 level determined in the alpaca in this study is lower than the lowest reported in the literature in domestic animals and is approximately 10% of that of the mean of horses evaluated by the same assays. Conversely, the levels of TxB_2 and PGE_2 and PGEM detected for horses in this study were comparable to previously published results, which indicates that the results are repeatable and that the camelid-specific species difference is likely real using these tests (Patrono *et al,* 1986).

Both species evaluated in this study had similar inducible COX-2 levels, measured by PGE_2 and metabolite analysis after induction with LPS (Patrono *et al,* 1986; Reinke and Brune, 1988 and Van Hoogmoed *et al,* 2004). PGE_2 is constitutively produced as a primary product of arachidonic acid metabolism via COX-2 enzymatic activity in multiple cells in the body as a result of external stimuli and activation of that pathway (Hamburg and Samuelsson, 1971). In whole blood assays, the source for COX-1 products (TxB_2) is platelets and the source of COX-2 products (PGE_2 and metabolites) is leukocytes. This methodology utilising whole blood has been generally accepted as the gold standard for determining COX-1/COX-2 enzyme activity specificity because it incorporates the proteins, cells, platelets and circulating enzymes into the assay that normally occur in circulating blood (Papich, 2008). These components are not present in isolated cells or enzyme systems.

In veterinary drug studies, commercially available ELISAs have largely replaced the more expensive and cumbersome radioimmunoassays and as a result have become the accepted standard for determining TxB_2 and PGE_2 (and metabolites) to ultimately evaluate COX-1 and COX-2 inhibition (Beretta *et al,* 2005; Brideau *et al,* 2001; Cunibeti *et al,* 2012; Davis *et al,* 2015; Duz *et al,* 2015; Minter *et al,* 2011). It needs to be noted that use of liquid chromatography–mass spectrometry (LC-MS) has been described for determining both TxB_2 and PGE_2 concentrations in serum, but these methodologies have largely been regulated to human studies only with few published studies in animals to date (Cao *et al,* 2011; Knych *et al,* 2015). The authors have an ongoing study developing LC-MS methodology for comparison with the results of this study. While the prostaglandin (PGE_2 and PGEM) levels were comparable to other domestic species, what was surprising with this study was the serum TxB_2 concentrations in alpaca that were not measurable with Assay 1a and were substantially lower than that of horses when measured with Assay 1b, indicating that the use of these specific bioanalytical assays to evaluate COX activity in this species is not appropriate.

The assays utilised to determine TxB_2, PGE_2 and PGEM in this study have been utilised in multiple animal species including domestic dogs, cats and horses indicating cross-species efficacy for these assays (Beretta *et al,* 2005; Brideau *et al,* 2001; Cunibeti *et al,* 2012; Duz *et al,* 2015). The fact that levels were detectable in horses and control values were as expected based on known standards indicate that there was no assay or human malfunction that resulted in the unusually low levels that were detected. Despite the history of use of ELISA in multiple species and validation through known standards, one variable with the multiple assays used in this study is the antibody bound to the wells of each 96 well plate. Assay 1a utilises a goat polyclonal anti-mouse IgG antibody bound to the individual well, while the Assay 1b utilises a mouse, monoclonal anti-rabbit IgG antibody. These both bind preferentially to a monoclonal rabbit antibody specific for TxB_2. These assays function based on the competition between TxB_2 and a TxB_2 acetylcholinesterase (AChE) conjugate for those limited well bound antibody sites. The AChE Tracer + TxB_2 conjugate is maintained at a constant level while the concentration of free TxB_2 varies. This allows for an inverse quantitation of serum TxB_2 levels. It is possible that there may be decreased binding of the free TxB_2 or AChE + TxB_2 conjugate to the TxB_2 specific antibody because of differences in the alpaca TxB_2 protein. While it may be possible, it was beyond the scope of this study to determine and quantitate any structural differences in alpaca TxB_2 Monoclonal anti-thromboxane antibodies, as was utilised in Assay 1b, have traditionally shown high specificity for TxB_2 with limited detectable cross reactivity (Reinke and Brune, 1988). Further studies are indicated, including specific mapping of the structure.

This is the first study of COX isoenzyme activity in alpaca the veterinary literature. The clinical implications in alpaca of low TxB_2 indicating low intrinsic COX-1 levels determined by these ELISAs are substantial. The low TxB_2 and subsequent COX-1 levels detected in this study imply that this assay may not be useful to assess NSAID COX isoenzyme inhibition in this species. In addition, if these results are real, the low levels of TxB_2 as an indicator of COX-1 activity may indicate a substantially higher risk of toxicity in alpaca for medications that inhibit these enzymes. NSAIDS favoring COX-1 inhibition, such as phenylbutazone and flunixin meglumine, are commonly administered and recommended for camelids with clinical disease (Drew *et al*, 1992; Navarre *et al,* 2001a; Navarre *et al,* 2001b). Further study is needed to determine the severity of this increased risk in alpaca. Pending further investigations, including verification of low TxB_2 levels using different analytical methods, such as LC-MS, practitioners may be advised to use caution when prescribing these medications, or others that affect COX isoenzymes.

The association between decreased COX-1 activity and gastric ulceration is well established in both humans and animals (Amagase *et al,* 2014; Rodriguez *et al,* 2010; Sinha *et al*, 2013; Jones and Budsberg, 2000). Gastric ulceration or ulceration of the glandular compartment of the stomach in the absence of administration of NSAIDs has been documented in camelids including alpacas (Cebra *et al,* 2003; Smith *et al,* 1994). To date, a definitive etiological agent has not yet been determined and there are few publications examining the effects of NSAIDS in alpacas or similar species. A study describing the association of prostaglandins with intestinal motility in llamas showed no response to NSAID drug exposure, but did not specifically investigate association of COX isoenzymes and the protective layer of the glandular gastric third compartment (C3) (Van Hoogmoed *et al,* 2004). The presence of these enzymes does not necessarily describe their specific activity. The association of constitutively expressed COX-1 with the protective prostaglandin layer of the glandular stomach leads to the conclusion that an animal with intrinsically low COX-1 levels is likely to be prone to gastric ulceration. This is concerning for all South American camelids, not just alpaca, because low levels of TxB_2 as an indicator of COX-1 observed in this study may be a potential predisposing factor for the C3 ulceration problems observed in these species (Cebra *et al,* 2003; Smith *et al,* 1994).

Further studies are indicated, including evaluation of gastric mucosal COX-1 levels to expand our knowledge base regarding COX enzymes in alpaca. This data is compelling in its implication that the unique physiology of the alpaca in respect to COX isoenzymes could affect NSAID metabolism and it may be a contributing or predisposing factor for gastric ulceration secondary to administration of these drugs. Further study of this same nature is also indicated in other camel and camelid species.

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DOI : 10.5958/2277-8934.2018.00035.8 Vol 25 No 3, p 261-265

SUPEROVULATION PROTOCOLS BEFORE OVUM PICKUP IN CAMEL

T. Ararooti, F. Panahi and A. Niasari-Naslaji
Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

ABSTRACT

The objective of this study was to investigate different superovulation treatments prior to ovum pick up in dromedary camel during non-breeding season. Follicular waves were synchronised following 2 GnRH injections 14 days apart. Two days after the 2nd GnRH (Day 0 of experiment), donors were assigned into 4 groups and received FSH (390 mg; n=4), eCG (2000 IU)-FSH (390 mg; n=5), eCG (3000 IU)-FSH (200 mg; n=6) and eCG (2000 IU)-FSH (160 mg; n=6). On the morning of day 4, donors were examined by ultrasonography to record ovarian follicles (≥4 mm in diameter). Data were analysed using GLM procedure followed by Tukey in SAS. Total number of follicles ≤6 mm was significantly greater in eCG (3000 IU)-FSH (200 mg; 6.2±0.95) than FSH (390 mg; 1.7±0.48) and eCG (2000 IU)-FSH (160 mg; 1.2±0.60; P<0.05). Total number of follicle >6 mm in eCG (3000 IU)-FSH (200 mg; 9.3±1.20) was less than FSH (390 mg; 17.2±1.18) and eCG (2000 IU)-FSH (390 mg; 14±1.18; P<0.05). There was no difference in total number of follicles between FSH (390 mg; 19±1.35), eCG (2000 IU)-FSH (390 mg; 17.4±0.93) and eCG (3000 IU)-FSH (200 mg; 15.5±1.75; P>0.05). In conclusion, superovulation with FSH (390 mg) and eCG (2000 IU)-FSH (390 mg) could provide relatively good number of follicles with suitable size for aspiration in ovum pick up program of dromedary camel.

Key words: Dromedary camel, eCG, FSH, OPU, superovulation

Reproductive efficiency of camel remained low due to inherent delay in maturity, short breeding season (Merkt *et al,* 1990), poor expression of estrous behaviour (Tibary and Anouassi, 1997), poor conception rate (Wilson, 1984), long gestation and inter-calving intervals (Merkt *et al,* 1990). Application of modern reproductive technologies allows shorting of generation interval and increases the number of progeny from elite male and female (McKinnon and Tinson, 1992; Skidmore *et al,* 1992). Although, *in-vitro* production of embryos (Khatir *et al,* 2004, 2007; Khatir and Anouassi, 2006) and cloning (Wani *et al,* 2009) have become a subject of interest for increasing the population of elite camels, the number of good quality oocyte is considered as an important limiting factor for successful IVP and cloning programs (Sartori *et al,* 2010; Leroy *et al,* 2011).

The application of ultrasound-guided follicular aspiration technique, also known as ovum pick-up (OPU), is a reliable, non-invasive and repeatable procedure for recovering oocytes in live animals. The objective of superovulation of donors prior to OPU is to increase the number of follicles suitable for puncture and to enhance and manage oocyte retrieval for embryo production (Chaubal *et al,* 2006). Superovulation of bovine donors with FSH prior to OPU, considering the proper interval between the termination of superovulation and OPU (Sirard *et al,* 1999), resulted in an increased proportion of cumulus-oocyte complexes (COCs) suitable for culture subsequent with higher blastocyst rate and numbers of embryos per OPU session (Vieira *et al,* 2014).

FSH, eCG and eCG-FSH were widely used for superovulation in embryo transfer programs in camel (Anouassi and Ali, 1990; Vyas *et al,* 1998; Skidmore *et al,* 2002; Anouassi and Tibary, 2013; Ararooti *et al,* 2017, 2018). However, there is not any protocol of superovulation prior to OPU in dromedary camel. The objective of this study was to find out the superovulation protocols that could increase the number of follicles suitable for puncture in dromedary camel.

Materials and Methods

Experimental animals

Dromedary camel donors (n=21), 6 to 14 years old and 504.52±15.02 kg, free from any visible uterine infection and ovarian abnormalities that could be detected by vaginoscopy, transrectal palpation and ultrasound examination, were used in this experiment during non-breeding season (April to June).

SEND REPRINT REQUEST TO A. NIASARI-NASLAJI email: niasarinaslajiamir@gmail.com

Ultrasonography

Females were restrained in a stanchion and all procedures were performed on standing position. Routine ultrasound examinations of ovaries and uterus were conducted, using a real time ultrasound scanner equipped with 7.5 MHz transducer. Ovarian structures including follicles (≥4 mm) were measured with internal caliper.

Experimental Design

Follicular waves were synchronised using 2 intramuscular administrations of GnRH analogue (Alarelin, 25 µg, Vetaroline®, Aburaihan, IRAN; Moghiseh *et al,* 2008) 12 to 15 days apart (Fig 1). Ultrasound scanning was performed prior to GnRH to ensure the presence of a mature follicle (13 to 17 mm in diameter) on the ovary (Nikjou *et al,* 2008). Two days after the 2nd GnRH injection (Day 0 of superovulation), donors were assigned into 4 treatment groups and received FSH (390 mg, Folltropin-V®, Bioniche, Canada; intramuscular; n=4), eCG (2000 IU, Pregnecol®, Bioniche, Canada)-FSH (390 mg; n=5), eCG (3000 IU)-FSH (200 mg; n=6) and eCG (2000 IU)-FSH (160 mg; n=6; Fig 1). Donors in FSH (390 mg) group, received twice daily FSH in decreasing doses (80, 50, 30, 20, 10 mg) between Days 0 and 4 of superovulation and single dose of FSH on Day 5 of superovulation (10 mg; Fig 1). Donors belonged to eCG (2000 IU)-FSH (390 mg) group, received a single intramuscular administration of eCG (2000 IU) on Day 0, followed by twice daily injections of FSH in decreasing doses (80, 50, 30, 20, 10 mg), beginning on Day 0 of superovulation, followed by a single injection of 10 mg FSH on Day 5 of superovulation (Fig 1). In eCG (3000 IU)-FSH (200 mg) group, donors received a single intramuscular administration of eCG (3000 IU) on Day 0, followed by twice daily injections of FSH in decreasing doses (40, 30, 20, 10; Fig 1). In eCG (2000 IU)-FSH (160 mg) group, eCG (2000 IU) was administered on Day 0, and twice daily injections of FSH in decreasing doses (30, 20, 20, 10), beginning on Day 0 of superovulation (Fig 1). On Day 4 of superovulation, camels received 2 doses of prostaglandin $F_{2\alpha}$ analogue (25 mg Dinoprost tromethamine; Lutalyse®, pfizer, Belgium), 12 hours apart and were examined by ultrasonography in the morning to record ovarian follicles (≥4 mm in diameter; Fig 1).

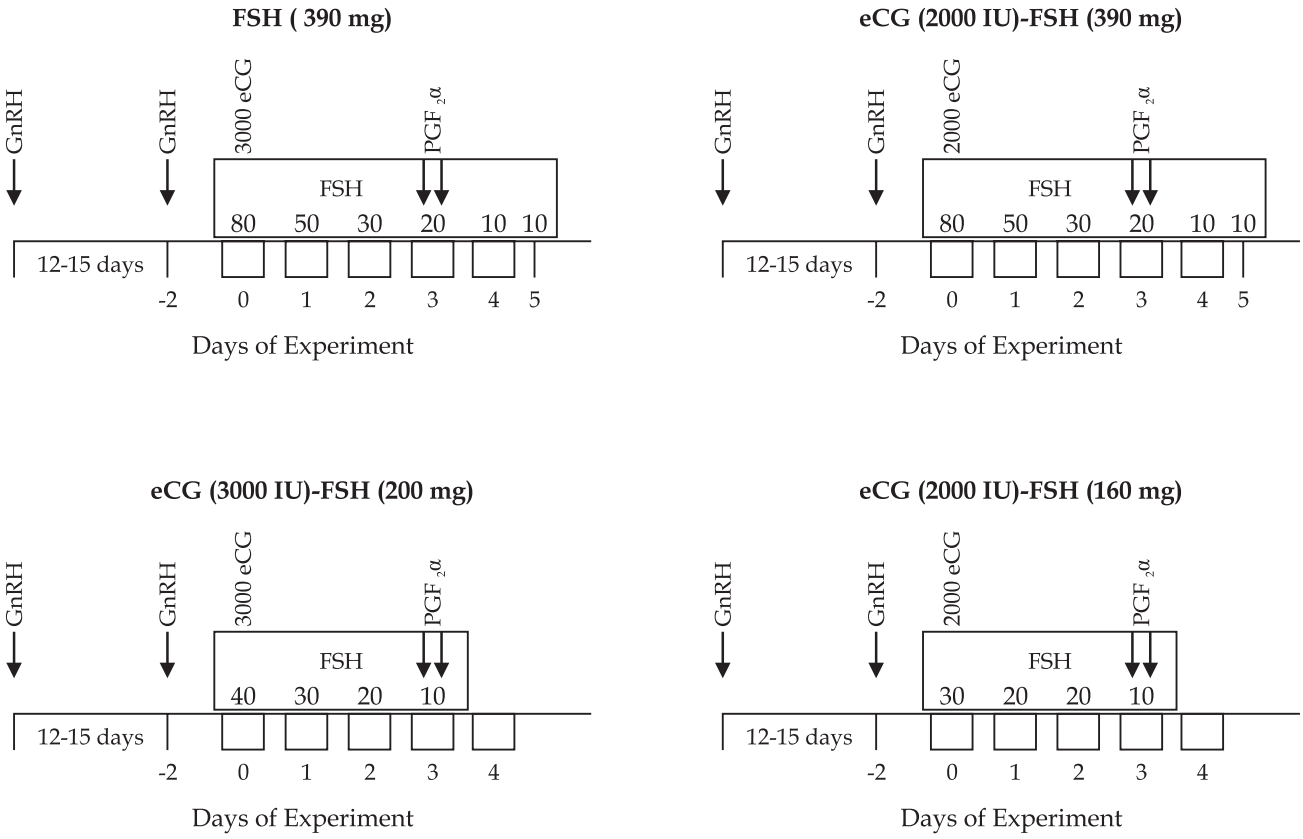

Fig 1. Experimental protocols for camels assigned into 4 superovulation protocols: FSH (390 mg); eCG (2000 IU)-FSH (390 mg), eCG (3000 IU)-FSH (200 mg) and eCG (2000 IU)-FSH (160 mg).

Statistical analysis

Between group differences were compared using Proc GLM followed by Tukey in SAS (SAS, 2014). If the assumptions of parametric tests were not achieved, the non-parametric ANOVA for single factor (Kruskal Wallis one-way ANOVA) was used. Data were presented as means±SEM.

Results and Discussion

At the time of the 1st and 2nd GnRH analogue injections, the size of ovarian follicle was 16.1±0.76 mm and 13.1±0.26 mm, respectively. On Day 4 of superovulation, average follicle diameter in FSH (390 mg; 8.1±0.27; 4.9-13.9 mm) and eCG (2000 IU)-FSH (390 mg; 7.7±0.23; 4-12.7 mm; P>0.05) was greater than eCG (3000 IU)-FSH (200 mg; 6.4±0.16; 4-10 mm; P<0.01). Follicle diameter in eCG (2000 IU)-FSH (160 mg; 7.4±0.33; 4-10.5 mm) was similar to other experimental groups (P>0.05). On Day 4 of superovulation, the number of follicles ≤6 mm in diameter in eCG (3000 IU)-FSH (200 mg; 6.2 ± 0.95; 3-9) was greater than FSH (390 mg; 1.7 ± 0.48; 1-3) and eCG (2000 IU)- FSH (160 mg; 1.2 ± 0.60; 0-3; P<0.05; Fig 2). The number of follicles >6 mm in diameter in FSH (390 mg; 17.2±1.18; 14-19) and eCG (2000 IU)-FSH (390 mg; 14±1.18; 10-17; P>0.05; Fig 2) groups were greater than eCG (3000 IU)-FSH (200 mg; 9.3±1.20; 5-14; P<0.05) and eCG (2000 IU)-FSH (160 mg; 3.5±0.76; 0-5; P<0.01; Fig 2). The total number of follicle was similar (P>0.05) in FSH (390 mg; 19±1.35; 15-21) and eCG (2000 IU)-FSH (390 mg; 17.4±0.93; 15-20) and eCG (3000 IU)-FSH (200 mg; 15.5±1.75; 12-23) and greater than eCG (2000 IU)-FSH (160 mg; 4.7±0.71; 3-8; P<0.001; Fig 2).

Single injection of eCG (1500 to 6000 IU) was the easiest and the first choice to superovulate she-camel (Anouassi and Ali, 1990; Cooper *et al,* 1990; McKinnon *et al,* 1994; Vyas *et al,* 1998; Tinson *et al,* 2000; Anouassi and Tibary, 2013). Following 1500 and 2000 IU eCG administration, 4.2 ± 1.12 follicles (range 0-10) and 5 ± 1.19 follicles (range 0-19) were detected, respectively (Anouassi and Ali, 1990). In another study, using 2500 IU eCG, 7.7 ± 0.3 follicles were achieved (Khalifa *et al,* 2016). The variation in superovulatory response following eCG treatment was evident and could be attributed to individual refractoriness to the hormone (Anouassi and Tibary, 2013). FSH has been successfully used for the superovulation in camel (McKinnon *et al,* 1994; Vyas *et al,* 1998). In more recent study, the average number of follicles was 17.7±3.40 following FSH (390 mg) treatment during breeding season (Ararooti *et al*, 2017) which was very similar to the result of the present study (19±1.35) considering the fact that it was conducted from April to June, during non-breeding season. This clearly indicates that even throughout the non-breeding season, it is possible to have good number of follicle for OPU in dromedary camel. Previous studies have shown that dromedary camel has growing follicle throughout the year (Nawito *et al,* 1968) and it is possible to run multiple ovulation and embryo transfer programs in dromedary camel during non-breeding season (Nowshari and Ali, 2005).

ECG is relatively inexpensive hormone and due to long half-life, single injection is sufficient for ovarian superovulation; whereas, FSH is much more expensive and due to its short half-life, multiple injections are necessary to achieve good ovarian superovulation (Monniaux *et al,* 1983). In order to use the long half-life of eCG while using the benefit of FSH, a combination of eCG and FSH has also been

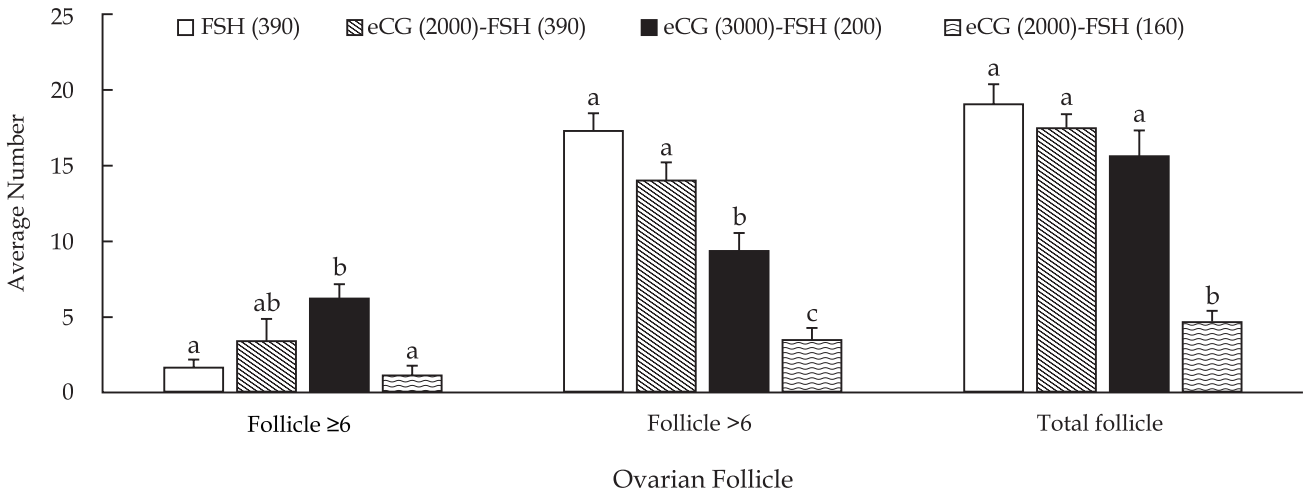

Fig 2. Number of follicles ≤6 mm and >6 mm and total number of follicles detected on Day 4 of super ovulation in dromedary camels with FSH (390 mg); eCG (2000 IU)-FSH (390 mg), eCG (3000 IU)-FSH (200 mg) and eCG (2000 IU)-FSH (160 mg).

applied for the superovulation in camel (Skidmore *et al,* 2002; Nowshahri and Ali, 2005; Nikjou *et al,* 2008; Skidmore and Billah, 2011; Ararooti *et al,* 2017; 2018). In an earlier study, using eCG (2500 IU) and FSH (400 mg), 19.7 ± 5.3 (4–35) follicles was recorded (Skidmore *et al,* 2002). Using nearly the same protocol (eCG: 2000 IU and FSH: 400 mg), 24.6 ± 2.92 (15–33) follicles was detected in bactrian camel (Nikjou *et al,* 2008). In a comparative study, superovulatory response based on the number of follicle in treated donors with eCG (2000 IU)-FSH (400 mg) was better than treated donors with FSH (400 mg; Nowshari and Ali, 2005). However, in another study, there was not any difference between treated donors with FSH (390 mg) and eCG (1000 IU)-FSH (330 mg) in the number of follicles (17.7±3.40 and 25.8±4.84, respectively; Ararooti *et al,* 2017). In present study, there was no difference in the number of follicles among FSH (390 mg; 19±1.35) and eCG (2000 IU)-FSH (390 mg; 17.4±0.93) groups (P>0.05).

Superstimulatory response in eCG (2000 IU)-FSH (160 mg) was lower than other treatments (total number follicle=4.7±0.71; P<0.001). This clearly indicates that certain amount of FSH is necessary besides eCG in order to achieve good superovulation.

The total number of follicles ≤6 mm in eCG (3000)-FSH (200 mg, 6.2±0.95) was significantly greater than FSH (390 mg, 1.7±0.48) and eCG (2000 IU)-FSH (160 mg, 1.2±0.60). Oocytes originated from large follicles (>6mm) were more competent than those collected from small follicles for IVF in camel (Khatir *et al,* 2007). In camel, the rate of nuclear maturation following IVM was slightly, but not significantly, greater for oocytes originated from large (>6 mm; 86%) than those from small follicles (3-6 mm; 73%; Khatir *et al,* 2007). In cattle, the rate of cleavage and the proportion of dromedary oocytes that developed to the blastocyst stage were also greater for oocytes obtained from large follicles compared with those originated from small follicles (Iwata *et al,* 2004; Lequarre *et al,* 2005). Conclusively, although the total number of follicles in FSH (390 mg), eCG (2000 IU)-FSH (390 mg) and eCG (3000 IU)-FSH (200 mg) protocols were similar (P>0.05) but the number of follicles >6 mm was lower in eCG (3000 IU)-FSH (200 mg), than 2 others protocols (P<0.05). It seems that an increase in the concentration of eCG could result in the second population of follicles, visible on Day 4 of superovulation. Accordingly, for eCG (3000)-FSH (200 mg) combination, it might be advised to postpone OPU, till follicles reach to suitable size for puncture (>6 mm). In conclusion, FSH (390 mg) and eCG (2000 IU)-FSH (390 mg) could provide relatively good number of follicles with suitable size for oocyte aspiration in dromedary camel.

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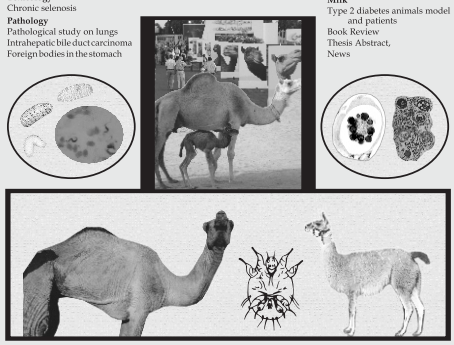

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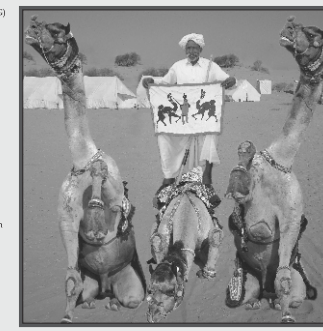

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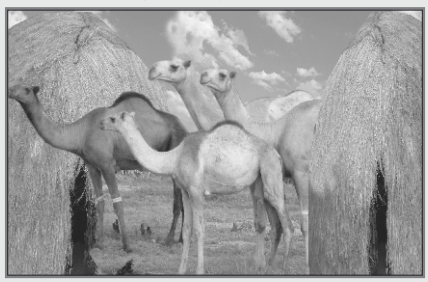

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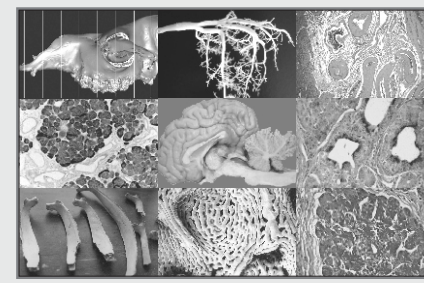

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DOI : 10.5958/2277-8934.2018.00036.X Vol 25 No 3, p 267-271

RELATIONSHIP BETWEEN THE SIZE OF THE PRE-OVULATORY FOLLICLES, SERUM CONCENTRATIONS OF OESTRADIOL AND PROGESTERONE, CORPUS LUTEUM DIAMETER AND PREGNANCY RATE IN CAMELS (*Camelus dromedarius*)

I.M. Ghoneim[1,2], M.M. Al-Eknah[1], M.I. Adam[3] and M.M. Waheed[1,2]

[1]Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Al-Hufof P.O. 400, Kingdom of Saudi Arabia

[2]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12515, Egypt

[3]Department of Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Khartoum, Sudan

ABSTRACT

Twenty-five clinically healthy female camels were used to investigate the relationship between pre-ovulatory follicle (POF) size, serum concentrations of oestradiol-17β (E_2) and progesterone (P_4) with pregnancy rate in dromedaries. Each sexual receptive animal experienced trans-rectal ultrasound examination before mating by proved fertile male (day 0) to monitor the number and diameter of POFs and 24, 48 and 72 hours post mating to detect the ovulation. Serum E_2 and P_4 concentrations were assessed on day 0, 1, 2, 3 and 14 post- and also on day 60 in proved pregnant animals. Results revealed that the mean diameter of POF was 14.09±0.69 mm (7.6-19.4 mm). POFs were identified in 44% (n=11) and 56% (n=14) without significant difference on the right and left ovaries, respectively. Each sexually receptive female displayed overt oestrous signs. Seventy-six (n=19) and 24 (n=6) per cent of ovulations occurred until 24 and between 24 and 48 hours post-mating, respectively. The pregnancy rate was 76 % (n=19). No significant correlation was recorded between POF diameter and pregnancy rate (r= -0.04). On day 0, the mean E_2 concentration of the pregnant animals (61.90±2.86 pg/ml) was not significantly different from that of non-pregnant animals (60.03±3.82 pg/ml). No significant correlations have been observed between POFs diameter and E_2 concentrations on the day 0 (r= 0.30). The mean P_4 concentration varied significantly ($P<0.05$) on the day 14 post-mating between pregnant (3.16 ± 0.09 ng/ml; range 2.51-3.54) and non-pregnant (0.38 ± 0.02 ng/ml; range 0.31-0.44) animals. There was a significant correlation ($r^2=0.56$, $P<0.05$) between the diameter of corpus luteum (CL) and P_4 concentrations. In conclusion, the POFs having diameters between 7.60 and 19.50 mm secrete enough E_2 to produce overt oestrous signs and possess the potential to ovulate.

Key words: Camels, oestradiol, corpus luteum pregnancy rate, pre-ovulatory follicle, progesterone

The dromedary camel is a seasonal breeder with a relatively short breeding season (Wilson, 1984; Tibary and Anouassi, 1997a). The females are induced ovulators, requiring copulation or ovulatory stimulus to induce the ovulatory process (El-wishy, 1987; Marie and Anouassi, 1987). If the ovulation is not induced, follicles may undergo regression of cystic formation (Skidmore *et al,* 2013). Camelids displayed complete sexual receptivity over a wide range of follicle sizes (Sumar *et al,* 1993; Ghoneim *et al,* 2015). Before ovulation, the follicles ought to attain the diameter of acquiring the ovulatory capacity (Tinson and McKinnon, 1992; Manjunatha *et al,* 2011). The failure of ovulation is one of the factors responsible for low conception rates in artificial insemination in dromedary camels (Deen *et al,* 2005). The inappropriate diameter of the follicle during the induction of ovulation might be reasons for reduced success of pregnancy. Furthermore, it was reported that the diameter of pre-ovulatory follicle has a positive effect on the size of post ovulation CL and conception in buffaloes (Rahman *et al,* 2012; Pandey *et al,* 2018). This study was planned to investigate the relationship between POFs size, serum profile of E_2 and P_4, CL diameter and pregnancy rate in the dromedary camels.

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Materials and Methods

Breeding care and management of experimental animals

The Scientific Research Deanship Ethics Committee, King Faisal University, licensed the experimental protocols. The study was conducted during the period between September 2016 and March 2017 (within the breeding season in Saudi Arabia). Twenty-five clinically healthy, non-lactating, non-pregnant camels with no reproductive disorders; multiparous, were used. Animals were between 8-15 years of age and weighing 450-600 kg. Camels were kept in pens at the Camel Research Centre, King Faisal University, Kingdom of Saudi Arabia (25.38°N latitude and 49.58°E longitude). The females were fed standard formula; salts and water were supplied a*d lib*. One vasectomised and four healthy male camels were kept next to females. During the experiment, all females were observed for oestrus signs (Mahla *et al,* 2015) and proved to be in oestrus by teasing twice daily (12 hours interval, each for 15 min) by a vasectomised camel without allowing mating. The sexually receptive females were subjected to natural mating by proved fertile male camel.

Ultrasound examination

Each sexual receptive animal underwent trans-rectal ultrasound examination using linear-array 7.5 MHz transducer (MyLab Five VET, Esaote, Italy; Tibary and Anouassi, 1996). Ultrasonography was performed immediately before mating (day 0) to monitor the number and diameter of POFs (Skidmore *et al,* 1996). The diameter of POF was measured at the widest point and at a right angle to the first measurement. According to the size of the POFs, animals were categorised into 3 groups, group I (small size follicles: ≤10 mm), group II (medium size follicles: >10 -15 mm) and group III (large follicles: >15–20 mm). Additionally, the ovaries were scanned 24, 48 and 72 h post-mating to confirm the ovulation (Skidmore *et al,* 1996) and on day 60 to confirm pregnancy and to measure the diameter of the CL gravidities (Vyas *et al,* 2004).

Blood Sampling

Immediately before mating (day 0), 1, 2, 3, 14 and 60 days post-mating, blood samples (20 ml) were collected by jugular venipuncture for P_4 and E_2 assay. The samples were centrifuged at 1400g for 15 min within 2 h of collection. The serum was separated and stored at -20°C until subsequent analysis.

Hormonal Analysis

Both E_2 and P_4 concentrations were assayed using ELISA kits (Cayman Chemical Company, Ann Arbor, MI 48108, USA). Procedures were carried out as recommended by the manufacturer of kits. Absorbance was measured using ELISA reader (Absorbance Microplate Reader ELx 800 and Microplate Strip Washer ELx800; BioTek; Highland Park, VT, USA), the standard curve was prepared using both E_2 and P_4 ELISA standard. All tests were performed in duplicate wells. Cayman's E_2 ELISA kit has been validated previously using extracted oestradiol-17β. The intra and inter assay coefficients of variations were 7.4% and 10.4%. The minimum detection limit for E_2 was 6.6 pg/ml. The intra and inter assay coefficients of variations of P_4 were 7.3% and 16.4% for P_4 respectively. The minimum detection limit for P_4 was 0.0078 ng/ml.

Statistical Analysis

Data of follicular diameter, E_2 and P_4 concentrations are presented as means ± SEM and compared by two way Analysis of Variance (ANOVA) and Correlations Coefficients (r^2) using SPSS statistical software (2013), version 22.0. (SPSS, 2013).

Results and Discussion

The study revealed that during the sexual receptivity, the diameter of POFs was between 7.60 and 19.50 mm, with mean diameter of 13.04 ± 0.70 mm (n=25). Table 1 shows the distribution of the sexual receptive animals on 3 follicular size groups as well as, the mean concentration of E_2 in each size group. The study revealed that 28% (n=7), 48% (n=12), and 24% (n=6) of the camels accepted mating while POF was between ≤ 10 mm, >10-15 mm and >15-20 mm in diameter, respectively. The difference in follicular diameters between female camel groups was highly significant (P<0.01).The concentration of E_2 on the day 0 was between 40.30 and 94.2 pg/ml and the mean concentration was 61.90±2.86 pg/ml. No correlation (r=0.30) was observed between POFs diameter and E_2 concentrations on day 0. Each sexually receptive female displayed overt oestrous signs. In all animals, only one POF was detected before mating. POFs were identified in 44% (n=11) and 56% (n=14) on the right and left ovaries, of the studied animals, respectively without significant difference. Ultrasound examination revealed that 76% (n=19) and 24% (n=6) of the ovulation occurred between the 24 and 48 hours post-mating, respectively. The pregnancy rates were 71.43% (n=5), 83.33% (n=10) and 66.67% (n=4) in groups I, II and III, respectively. The total pregnancy rate was 76% (n=19). All foetuses were developed in

the left uterine horn by the day 60 of pregnancy. No significant correlations were recorded between POF diameter and pregnancy rate. In the pregnant animals, E_2 concentrations decreased steadily from the day 0 (58.91 ± 2.92 pg/ml) to 30.81 ± 0.53 pg/ml on day 14 post-mating and then elevated again on day 60 after mating to 39.20 ± 2.15 pg/ml. On the day 0, the mean E_2 concentration of the pregnant animals (58.91 ± 2.92 pg/ml) was not significantly different from that of non-pregnant animals (59.87 ± 1.71 pg/ml; Table 2). In the pregnant animals, P_4 concentration showed a gradual increase from the day one post-mating (0.35 ± 0.02 ng/ml) and reaches the maximum on day 60^{th} of gestation (5.21 ± 0.13 ng/ml). On other hand, in non-pregnant females, mean P_4 concentrations increased gradually till day 3 post-mating (0.59 ± 0.02 ng/ml) but dropped on the day 14^{th} post-mating (0.39 ± 0.04 ng/ml). The mean P_4 concentration varied significantly ($P<0.05$) on the day 14^{th} post-mating between pregnant and non-pregnant animals (Table 2). There was a significant correlation ($r^2=0.53$, $P<0.05$) between the diameter of CL and P_4 concentrations on day 60^{th} of pregnancy. However, retrospective analysis revealed no correlation (r=0.23) between the diameter of POF and that of CL on day 60^{th} of pregnancy.

There are dearth studies regarding pregnancy rates in dromedary camels (Nagy *et al,* 2005; Skidmore and Billah, 2006; Khatir *et al,* 2007). The wide range of follicular size for the period of the sexual receptivity reported in this study was also confirmed by other studies in dromedaries (Tibary and Anouassi, 1996; Musa and Abusineina, 1978) and in alpacas (Sumar *et al,* 1993). Similar with other reports (Musa, 1979; Tibary and Anouassi, 1997b), both ovaries are equally active. In contrast to our results, double, triple and quadruple ovulations have been documented in camelids (Tibary and Anouassi, 1996; Campbell *et al,* 2015). As the ovulation is verified by the existence of a corpus luteum 3 days post copulation (Fernandez-Baca *et al,* 1970), no one of the studied animals failed to ovulate. This is probably because most follicles were closed to the diameter of acquiring the ovulatory capacity (Tinson and McKinnon, 1992; Manjunatha *et al,* 2011). The ovulation induced within 48 hours post-mating which is consistent with the earlier observations (Marie and Anouassi, 1987; Manjunatha *et al,* 2011).The high concentration of E_2 verified during the day 0 is responsible for the behavioural oestrus (Homeida *et al,* 1988). The current study revealed that POFs size did not influence circulating concentrations of E_2 which is matched with the earlier study in cattle (Perry *et al,* 2005). On the contrary, other studies (Khalil, 1989; Pandey *et al,* 2011) reported correlation between the E_2 and follicular size. The decreased concentrations of E_2 during the first two weeks of gestation could be ascribed to ovulation and formation of the corpus luteum. The elevation of E_2 concentrations at day 60 of pregnancy is probably due to development of couple of ovarian follicles during pregnancy (El-Shahat *et al,* 2013) as the follicular activity is not inhibited in dromedary camels until 6 months of gestation (Tibary and Aonuassi, 1997a). Similar to the other reports (El-wishy, 1988; Ratto *et al,* 2011), this study recorded that all pregnancies developed in the left uterine horn. This phenomenon is attributed to specific surface molecule expression on the uterine epithelium allowing embryo apposition and adhesion only in restricted areas (Olivera *et al,* 2003). In disagreement with the results in cattle (Perry *et al,* 2007) and buffaloes (Rahman *et al,* 2012; Pandey *et al,* 2011), the present study shows no significant correlation between ovarian pre-ovulatory follicles diameters and pregnancy rate. It was reported that the animals with extreme small or large follicles are

Table 1. Distribution of the sexual receptive animals according to the follicular size and their serum oestradiol-17β concentrations.

Animal groups (Follicular diameter mm)	No. of animal	Mean diameter of follicle (mm)	Mean oestradiol-17β (E_2) level (pg/ml)	Conception rate (%)
Group I (7-10 mm)	7	8.44^a ± 0.30 [7.6 – 10.0]	51.63 ± 2.37 [43.2 – 59.7]	71.43
Group II (>10 -15 mm)	12	13.15^b ± 0.39 [10.7 – 14.6]	64.28 ± 3.77 [40.3 – 94.2]	83.33
Group III (>15–20 mm)	6	17.75^c ± 0.66 [15.7 – 19.5]	62.87 ± 4.64 [51.4 – 83.7]	66.67
P values	–	P<0.01	–	–

Means with different superscripts in the same column are significantly different at P<0.05 and P<0.01.

Table 2. Oestradiol-17β and progesterone concentrations on day 0 and 14 post-mating in pregnant and non-pregnant female camels.

Hormones	Pregnant female camels (n=19)	Non pregnant female camels (n=6).
Oestradiol-17β level pg/ml) on day 0	58.91 ± 2.92 [40.30 – 94.20]	59.87 ± 1.71 [52.40 – 64.20]
Progesterone (P_4) level (ng/ml) on day 14	3.16 ± 0.09^a [2.51 – 3.55]	0.38 ± 0.02^b [0.32 – 0.45]

Means with different superscripts in the same row are significantly different at P<0.001.

failed to conceive (Perry *et al,* 2007). In a previous study (Skidmore and Billah, 2006), pregnancy rates were between 43% and 53%. The high ovulation rates documented in the present work may explain the high pregnancy rate reported in this study. The failure of ovulation is the main factor responsible for low conception rates in dromedary camels (Deen *et al,* 2005). During this study, 6 female camels have failed to become pregnant probably due to failure of fertilisation or failure of embryo survival following successful fertilisation. The failure of conception in female camel has been assigned to uterine and/or uterine tube infection (Tibary *et al,* 2006). The CL is the transient source for P_4 during the pregnancy in dromedary camels (Al-Eknah *et al,* 2001). The reported distinct progesterone concentration in the pregnant camels on the day 14 post-mating makes it a diagnostic aid for pregnancy. The recorded low concentration of progesterone on day 14 in non-pregnant animals indicates the regression of the CL before this time (Marie and Anouassi, 1987; Tibary and Anouassi, 1996). It was also documented that the non-pregnant-mated camels has a short luteal phase (Agarwal *et al,* 1991; Deen *et al,* 2007). The significant correlation coefficients reported between the diameter of CL and P_4 concentrations on day 60^{th} of pregnancy, have been also confirmed in cattle (Vasconcelos *et al,* 2001; Machado *et al,* 2008; Bisinotto *et al,* 2012) and buffaloes (Rahman *et al,* 2012). In conflict with the results in cattle (Wecker *et al,* 2012), this study couldn't positively relate the follicle and CL sizes.

It was concluded that the POFs having diameters between 7.60 and 19.50 mm secrete enough E_2 to produce overt oestrous signs and possess the potential to ovulate. The diameters of such POFs didn't play a role on pregnancy rate and the resultant CL diameter.

Acknowledgements

The Scientific Research Deanship, King Faisal University, Kingdom of Saudi Arabia funded this study; (Project# 150038).The authors thank the personnel of the Camel Research Centre, King Faisal University, Saudi Arabia. Al-Ahsa 31982, Al-hufof P.O. 400 for their assistance in this project.

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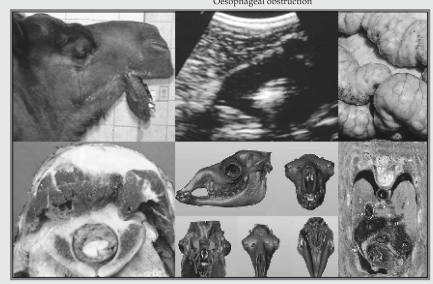

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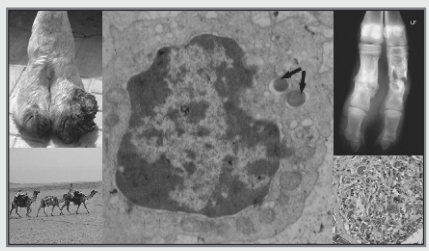

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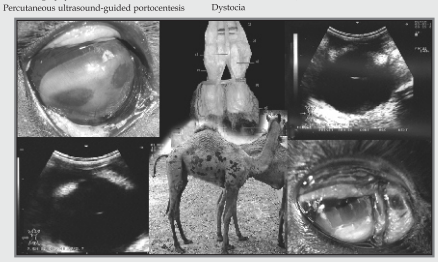

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DOI : 10.5958/2277-8934.2018.00037.1 Vol 25 No 3, p 273-274

Short Communication

ZOONOTIC DISEASES OF CAMELIDS – AN OVERVIEW WITH SPECIAL REFERENCE TO Q-FEVER AND BRUCELLOSIS

U Wernery and M Rodriguez Caveney
Central Veterinary Research Laboratory, Dubai, United Arab Emirates

An overview is presented of the most important zoonotic viral, bacterial, fungal and parasitic diseases of camelids followed by a closer look at Q-Fever and Brucellosis. As the zoonotic pathogens do often not produce a disease in camelids, infection instead of disease is mentioned in this present context.

Viral diseases

Eight viral diseases are listed in Table 1, of which camelpox, parapox, influenza and MERS have been described in humans after contact with camels (Wernery *et al,* 2014; Wernery, 2017; Fowler, 2010; Curasson, 1947; Manefield and Tinson, 1996 and Higgins, 1986). Rabies, Borna, Rift Valley Fever and West Nile Fever infections were described in camelids but no transmission to humans have been reported.

Table 1. Viral diseases causing infections in camelids and human by contact.

Disease	Occurrence			
	Dromedary	Bactrian	NWC's	Human
Rabies	+	+	+	–
Borna	+ Antibodies	–	+	–
Camelpox	+	+	**Experiment**	+
Parapox (Orf)	+	+	+	+
Rift Valley Fever	+	–	+	–
West Nile Fever	+	+	+	–
Influenza	–	+	–	+
MERS	+	–	–	+

Bacterial Diseases

Sixteen bacterial diseases are listed in Table 2 of which anthrax, plague, salmonellosis, brucellosis and dermatophilosis have caused disease in humans. References are the same as listed under Viral diseases.

Fungal Diseases

Reports of camelid fungal infections causing disease in humans are scanty (Table 3). However, it is known that skin diseases are caused by dermatophytes Microsporum and Trhicophyton and camel milk may contain aflatoxin (Wernery *et al,* 2014).

Table 2. Bacterial diseases causing infections in camelids and human.

Disease	Occurrence			
	Dromedary	Bactrian	NWC's	Human
Anthrax	+	+	+	+
Botulinum	+	–	–	–
Tetanus	+	+	+	–
Glanders	+	+	–	–
Melioidosis	+	-?	+	–
Plaque	+	+	–	+
Leptospirosis	+	+	+	–
Q-Fever	+	?	–	–
Salmonellosis	+	+	+	+
Paratuberculosis	+	+	+	?milk
Tuberculosis	+	+	+	–
Brucellosis	+	+	+	+
Chlamydiosis	?	?	+	–
Caseous Lymphadenitis	+	+	+	–
Listeriosis	+	?	+	–
Dermatophilosis	+	?	+	+

Parasitic Diseases

Out of eight parasitic infections in camelids, mange is the only one which have caused clinical lesions in human with certainty (Table 4). People contract the disease through contact with infected animals and develop the clinical picture of pseudoscabies (Wernery *et al,* 2014). Giardiosis,

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cryptosporidiosis and tick infestations are possible to occur in humans, but have not been described.

Table 3. Fungal disease causing infections in camelids and human.

Disease		Occurrence			
		Dromedary	Bactrian	NWC's	Human
Dermatophytes	Microsporum	+	+	+	+
	Trichophyton	+	+	+	+
Coccidiodomycosis		-	-	+	-
Mycotoxins -Aflatoxin -Ochratoxin -Zearalenone		+ Milk	?	?	+

Table 4. Parasitic diseases causing infections in camelids and human.

Disease	Occurrence			
	Dromedary	Bactrian	NWC	Human
Toxoplasmosis	+	+	+	–
Giardiosis	+	+	+	Possible (+) No published cases
Cryptosporidiosis	+	?	+	Possible (+) No published cases
Fasciolosis	+	+	+	–
Hydatidosis	+	+	?	–
Ticks	+	+	+	? No published cases
Mange	+	+	+	+
Wohlfahrtia	+	+	Fly is not present in NWCs	–

Q-Fever

Q-Fever caused by *Coxiella burnetii* is a zoonosis. The disease is distributed worldwide with the exception of New Zealand. In more than half of the cases, the infection in humans is asymptomatic. *Coxiella burnetii* can infect a broad spectrum of animal species and antibodies against QF have been detected in camelids from many countries. No QF disease has been observed in camelids and the pathogen has not been isolated from raw milk (Wernery, 2011).

Brucellosis

Human brucellosis remains one of the most common diseases with more than 500,000 new human cases annually. Infection prevalence in animal reservoirs determines the incidence of human cases because of human-animal interactions. Serological tests have now been validated for use in camelids and recent investigations found only 2 serological tests suitable (Soellner *et al,* 2018). Enriched culture is the gold standard for a proper diagnosis of brucellosis as PCR is not sensitive enough when testing camelid samples, which are not enriched.

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DOI : 10.5958/2277-8934.2018.00038.3 Vol 25 No 3, p 275-287

RAPID DETECTION OF ADULTERATED CAMEL MILK USING LOW-FIELD NUCLEAR MAGNETIC RESONANCE

Dalai Siren[1], Mingliang[2] and Rimutu Ji[1,2]

[1]Inner Mongolia Institute of Camel Research, Inner Mongolia, Badanjiran 737300, China
[2]Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, Inner Mongolia Agricultural University, Hohhot 010018, China

ABSTRACT

In this study, the transverse relaxation time (T_2) of adulterated camel milk obtained from different regions was detected using nondestructive low-field nuclear magnetic resonance (NMR). Camel milks from Alashan and Xinjiang regions, China were selected and treated under different conditions, including pasteurisation (65°C, 30 min) and high-temperature short-time (HTST) treatment (75°C, 15–20s) and adulterated with substances such as cow milk, sheep milk, soybean milk, urea and water. These adulterated milk samples were then subjected to low-field NMR detection and echo peak point data were recorded. The results showed that the distribution of the adulterated samples was well differentiated on the principal component score map and that characteristics among the samples were also clearly expressed by the principal component axis. Similarly, the T2 of the adulterated samples gradually changed with varying adulteration ratios, the characteristics of which were fully expressed. The present data indicated that low-field NMR was advantageous for high-accuracy, simple, rapid, nondestructive and on-site detection. This newly developed measuring method is more convenient and particularly suitable for the identification of adulterated camel milk compared with traditional detection methods.

Key words: Adulteration, camel milk, nuclear magnetic resonance, PCA method

Dairy products can provide various important nutrients that help maintain human health. High quality standards are applied to dairy products during processing, transportation and sale to protect consumer health. Among these standards, the safety and quality control of raw milk is most important, because any problem with the raw milk will directly affect all resulting products (Guo, 2004). In recent years, there have been many incidents concerning the safety of dairy products in China. Many substances are added to raw milk to increase profits. A typical example is the melamine incident, which caused serious physical and mental harm to the consumers.

Methods for detecting adulterated milk are mostly limited to traditional detection methods, including sensory, physical and chemical detection methods (Chen *et al,* 2007). However, these detection methods lead to dairy safety problems owing to disadvantages caused by complicated operations, such as a low separation rates and significant sample damage (GB 5408-5425, 1985). Furthermore, most traditional detection methods can only detect specific substances. Adulteration is particularly difficult to detect when the identity of the adulterant is unclear or there is no effective detection method. As a fast, simple and nondestructive detection method, low-field NMR analysis (Chen *et al,* 2006 and Fan *et al,* 2004) can measure interactions between water and other components in milk by detecting the transverse relaxation time (T_2), which indicates the state of water molecules in raw milk.

Camel milk is an emerging industry in China. Although commercial camel milk production is relatively new and has developed slowly, steps must be taken to prevent fake milk sources from entering the camel milk market. Compared with traditional detection methods, which involve complicated operations and poorly reflect the degree of adulteration, low-field NMR is more convenient, especially for the identification of fake camel milks. In this study, the transverse relaxation time (T_2) of adulterated camel milk obtained from different regions was detected using nondestructive low-field nuclear magnetic resonance (NMR).

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Materials and Methods

Camel milks from the Alashan and Xinjiang regions, China, were selected and cow milk, goat milk, water, soybean and urea were used to prepare adulterated camel milk. A NMI20 NMR imager and analyser Shanghai Newmai Electronic Technology Co., Ltd. was used in this experiment with the following main performance indicators: Magnetic field intensity, 0.5 T; magnetic pole gap, 35 mm; resonance frequency, 21–23 MHz; magnetic pole stability, after 4–5 h; evenness degree of magnetic pole, 2.5 ppm (12 mm × 12 mm × 12 mm); magnetic pole diameter, 165 mm. Other equipment used included a C-MAG MS4 magnetic stirrer (Thermo Fisher Scientific), AR2140 electronic scale (US OHAUS), thermostat water bath (Shanghai Yiheng Technology Co., Ltd), pipette (Eppendorf International Trade Co., Ltd) and FA25 high shear dispersing emulsifier (Yuhua Instrument Co., Ltd).

Camel milks were added cow and goat milks in ratios of 10, 20, 30, 40, 50 and 60%, which were subjected to NMR analysis (repeated 4 times). The prepared milk samples were pasteurised (65°C, 30 min) and subjected to NMR analysis (repeated 4 times). These milk samples were also subjected to high-temperature–short-time (HTST) processing (75°C, 15–20s) followed by NMR analysis (repeated 4 times). Water was added to camel milk at ratios of 2, 4, 6, 8% and subjected to NMR analysis (repeated 4 times). The lower detection limit of the water content was then determined. Soybean milk was also added to camel milk at ratios of 10, 20, 30, 40, 50 and 60% and then subjected to NMR analysis (repeated 4 times). Finally, urea was added to camel milk at different ratios and subjected to NMR analysis (repeated 4 times).

Sample detection

In the low field NMR software, the flame ionisation detector (FID) sequence was used to determine the centre frequency of the proton (^{1}H) nuclear magnetic resonance signal and the 90° pulse width in the sample. The Carr–Purcell–Meiboom–Gill (CPMG) sequence parameters were then set before each measurement was performed. Finally, the echo peak value was recorded. Each sample was automatically detected 3,000 times (measured within 0.5s).

Low-field NMR parameters

FID sequence parameters: P1 (90° pulse time), 25.5 μs; SW (sampling frequency), 100.0 kHz; SF1, 22 MHz; O1, 871.410 kHz; NS (cumulative number), 4. Sequence frequency (SF1) and Offset (O1) represent the centre frequency and O1 needs to be readjusted for each run.

CPMG sequence parameters: D1 (90° and 180° pulse interval time), 1200 μs; D2 (180° and 180° pulse interval time), 2400 μs; D0 (pulse repeat time), 3000 ms; SW, 100 kHz; NS, 4.

Data processing and analysis

Principal component analysis (PCA) was used to process and analyse echo peak data from the low-field NMR CPMG sequences of adulterated camel milk samples. PCA was performed using SPSS 19.0 software.

Results and Discussion

The CPMG echo peaks of different samples are shown in Figs 1 and 2. Differences were observed between the curvature and signal intensity of the CPMG echo peak maps of pure camel milk and camel milk adulterated with different substances. However, the differences between samples were not obvious. The PCA method was applied to these results.

In the PCA results (He, 2004), the CPMG echo peak data of the sample represents one point on the principal component score map, with the area formed by several points representing the overall quality differences among samples, which were characterised by conciseness and clarity. On the PCA score map, the total contribution rate can retain the ratio of the original data and the statistical method can be used when the total contribution rate is 70–85% (Xu and Wang, 2006). A higher contribution rate means that the main component better reflects data from the original indicators.

Results and analysis of adding cow milk to camel milk

Adding cow milk to Xinjiang camel milk

Xinjiang camel milk samples with added cow milk were subjected to low-field NMR measurements and PCA was performed on the resulting CPMG echo peak data. Each circle in the figure 3 represents the overall characteristics of milk samples with different ratios, with 4 marker points representing the 4 gradient repeats of the sample. A smaller distance between the 2 circles represents a smaller difference between the quality characteristics of these samples. Principal components 1 and 2 (pc1 and pc2) retained 97.95% of the original data. Samples were well differentiated on the pc axes. As the proportion of cow milk added increased, the distribution of the samples shifted from the bottom to the top of the pc2 axis and the mixed

samples became increasingly different to pure camel milk (Fig 3). The distributions of pure camel milk and the mixed samples containing 10% cow milk partially overlapped. This indicated that the lower limit of NMR detection for camel milk adulterated with cow milk was 10%. As shown in Table 1, the transverse relaxation time (T_2) of the mixed samples gradually increased with the increasing proportion of cow milk. The characteristics of this relaxation time are fully expressed on the principal component score map.

Furthermore, for the pasteurised samples, pc1 and pc2 retained 99.80% of the original data, with the results similar to those of unpasteurised samples (Fig 4, Table 1).

For the HTST-treated samples (75°C, 15-20s), pc1 and pc2 retained 99.80% of the original data. As the proportion of added cow milk increased, the distribution of the samples shifted from left to right on the pc1 axis and the mixed samples became increasingly different to pure camel milk. The distributions of pure camel milk and mixed samples containing 10% cow milk did not overlap (Fig 5, Table 1).

Adding cow milk to Alashan camel milk

As shown in Fig 6, for Alashan camel milk samples with added cow milk, pc1 and pc2 retained 97.95% of the original data. As the proportion of cow milk added increased, the distribution of the samples shifted from the top to the bottom of the pc2 axis and the mixed sample became increasingly different to pure camel milk. The distributions of pure camel milk and mixed samples containing 10% cow milk did not overlap.

Table 2 shows that the T_2 of the mixed samples gradually increased with an increasing proportion of added cow milk. For pasteurised samples, pc1 and pc2 retained 99.63% of the original data. As the proportion of added milk increased, the distribution of the samples shifted from left to right on the pc1 axis and the mixed samples became increasingly different to pure camel milk. The distributions of camel milk and mixed samples containing 20% cow milk partially overlapped (Fig 7, Table 2).

For HTST-treated samples (75°C, 15–20s), pc1 and pc2 retained 99.89% of the original data. As the proportion of added cow milk increased, the distribution of the samples shifted from left to right on the pc1 axis and the mixed sample became increasingly different to pure camel milk. The distributions of pure camel milk and the mixed samples did not overlap (Fig 8, Table 2).

Results and analysis of adding goat milk to camel milk

Adding goat milk to Xinjiang camel milk

Low-field NMR measurements of Xinjiang milk adulterated with goat milk showed that pc1 and pc2 retained 97.95% of the original data. The samples were well differentiated on the pc axes. However, as shown in Table 10, the T_2 values of these mixed samples gradually decreased with the increasing

Table 1. T_2 values of Xinjiang camel milk (CM), pasteurised camel milk (PCM) and HTST camel milk (HTST) samples adulterated with cow milk (Cow M), pasteurised cow milk (Cow PM), HTST cow milk (HTST Cow).

Milk Sample	10% cow milk	20% cow milk	30% cow milk	40% cow milk	50% cow milk	60% cow milk	Cow milk
79.33±0.31 (CM)	80.4±0.55	82.07±0.25	85.27±0.44	86.3±0.34	88.7±0.45	91.02±0.34	101.94±0.25 (Cow M)
77.41±1.06 (PCM)	78.03±0.69	79.87±0.29	82.42±0.87	85.1±0.29	86.3±0.16	88.11±0.22	101.76±0.26 (Cow PM)
83.00±0.73 (HTST)	79.29±0.43	81.1±0.28	83.13±0.49	85.55±0.09	87.35±0.11	–	107.2±1.23 (HTST Cow)

Data shown are averages.

Table 2. T_2 values of Alashan camel milk (CM), pasteurised camel milk (PCM) and HTST camel milk (HTST) samples adulterated with cow milk (Cow M), pasteurised cow milk (Cow PM), HTST cow milk (HTST Cow).

Milk Sample	10% cow milk	20% cow milk	30% cow milk	40% cow milk	50% cow milk	60% cow milk	Cow milk
96.61±0.67 (CM)	97.5±0.27	98.62±1.04	99.99±0.25	100.98±0.17	102.24±0.43	103.81±0.33	110.91±0.25 (Cow M)
93.48±2.67 (PCM)	91.69±0.41	93.71±0.38	95.68±0.67	96.91±0.08	97.24±0.43	98.96±0.68	107.84±0.67 (Cow PM)
97.44±0.83 (HTST)	91.93±0.27	94.25±0.56	96.01±0.32	98.3±0.43	99.03±0.13	–	110.01±0.68 (HTST Cow)

proportion of goat milk added, which was different to that observed for cow milk adulterated samples (Fig 9, Table 3).

The pc1 and pc2 retained 99.77% of the original data for pasteurised goat-milk-adulterated samples, and the testing results were similar to those of pure camel milk (Fig 10, Table 3).

The pc1 and pc2 retained 99.88% of the original data for HTST-treated (75°C, 15–20s) goat-milk-adulterated samples. As the proportion of goat milk added increased, the distribution of the samples shifted from right to left on the pc1 axis and the mixed samples became increasingly different to pure camel milk (Fig 11, Table 3).

Adding goat milk to Alashan camel milk

The pc1 and pc2 retained 99.95% of the original data on the PCA score map for Alashan camel milk samples adulterated with goat milk. The samples were well differentiated on the pc axes (Fig 12, Table 4).

The pc1 and pc2 retained 99.81% of the original data for pasteurised goat-milk-adulterated samples and the results of testing were similar to those of pure camel milk (Fig 13, Table 4).

The pc1 and pc2 retained 99.71% of the original data for HTST-treated (75°C, 15–20s) goat-milk-adulterated samples. As the proportion of goat milk added increased, the distribution of the samples shifted from right to left on the pc1 axis and the mixed sample became increasingly different to pure camel milk (Fig 14, Table 4).

Results and analysis of adding soybean milk to camel milk

Adding soybean milk to Xinjiang camel milk

The pc1 and pc2 retained 99.93% of the original data, as shown in Fig 15 for Xinjiang camel milk samples adulterated with soybean milk. As the proportion of soybean milk added increased, the distribution of the samples shifted from left to right on the pc1 axis. The distributions of pure camel milk and mixed samples containing 10% soybean milk did not overlap. Table 5 shows that the T_2 values of the mixed samples gradually increased with the increasing proportion of added soybean milk.

Adding soybean milk to Alashan camel milk

The pc1 and pc2 retained 99.92% of the original data, as shown in Fig 16 for Alashan camel milk samples adulterated with soybean milk. As the proportion of soybean milk added increased, the distribution of the samples shifted from left to right on the pc1 axis and the mixed sample became increasingly different to pure camel milk. The distributions of pure camel milk and mixed samples containing 10% soybean milk partially overlapped. Table 5 shows that the T_2 values of the mixed samples gradually increased with the increasing proportion of added soybean milk. However, in this experiment, the distribution was slightly uneven when a 10% soybean milk was added to the samples, which might be related to instability during machine operation.

Table 3. T_2 values of Xinjiang camel milk (CM), pasteurised camel milk (PCM) and HTST camel milk (HTST) samples adulterated with goat milk (Goat M, Goat PM, HTST Goat).

Milk Sample	10% goat milk	20% goat milk	30% goat milk	40% goat milk	50% goat milk	60% goat milk	Goat milk
79.33±0.31 (CM)	75.05±0.15	70.69±0.08	68.77±0.19	67.02±0.30	65.16±0.27	62.54±0.22	55.36±0.1 (Goat M)
77.41±1.06 (PCM)	73.45±0.12	71.05±0.27	68.59±0.12	66.14±0.14	64.17±0.52	62.77±0.44	56.14±0.34 (Goat PM)
83.00±0.73 (HTST)	72.15±0.28	71.04±0.28	68.07±0.15	64.98±0.12	62.85±0.31	–	57.01±0.35 (HTST Goat)

Table 4. T_2 values of Alashan camel milk (CM), pasteurised camel milk (PCM) and HTST camel milk (HTST) samples adulterated with goat milk (Goat M, Goat PM, HTST Goat).

Milk Sample	10% goat milk	20% goat milk	30% goat milk	40% goat milk	50% goat milk	60% goat milk	Goat milk
96.61±0.67 (CM)	85.71±0.65	79.47±0.13	73.34±0.26	68.69±0.14	64.29±0.14	60.68±0.23	49.84±0.18 (Goat M)
93.48±2.67 (PCM)	83.88±0.34	78.51±0.69	73.33±0.29	66.54±0.37	65.04±0.47	64.56±0.79	48.99±0.02 (Goat PM)
97.44±0.83 (HTST)	82.82±0.55	77.5±0.29	71.73±0.23	67.43±0.31	64.77±0.42	–	50.31±0.13 (HTST Goat)

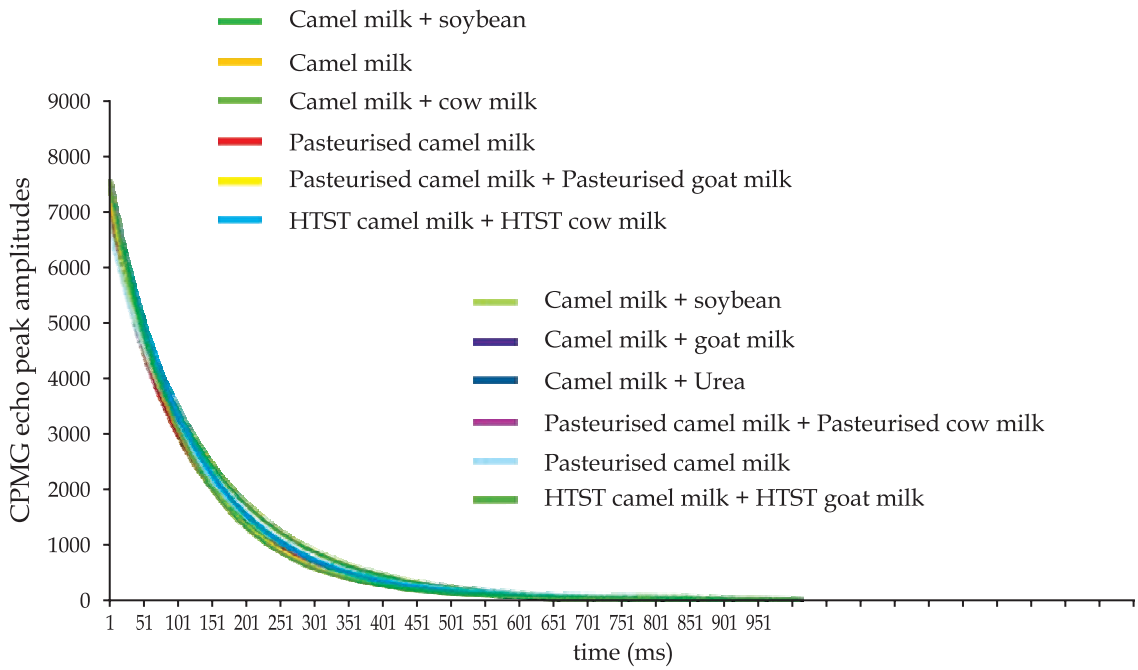

Fig 1. CPMG echo peak amplitudes of Xinjiang adulterated camel milk.

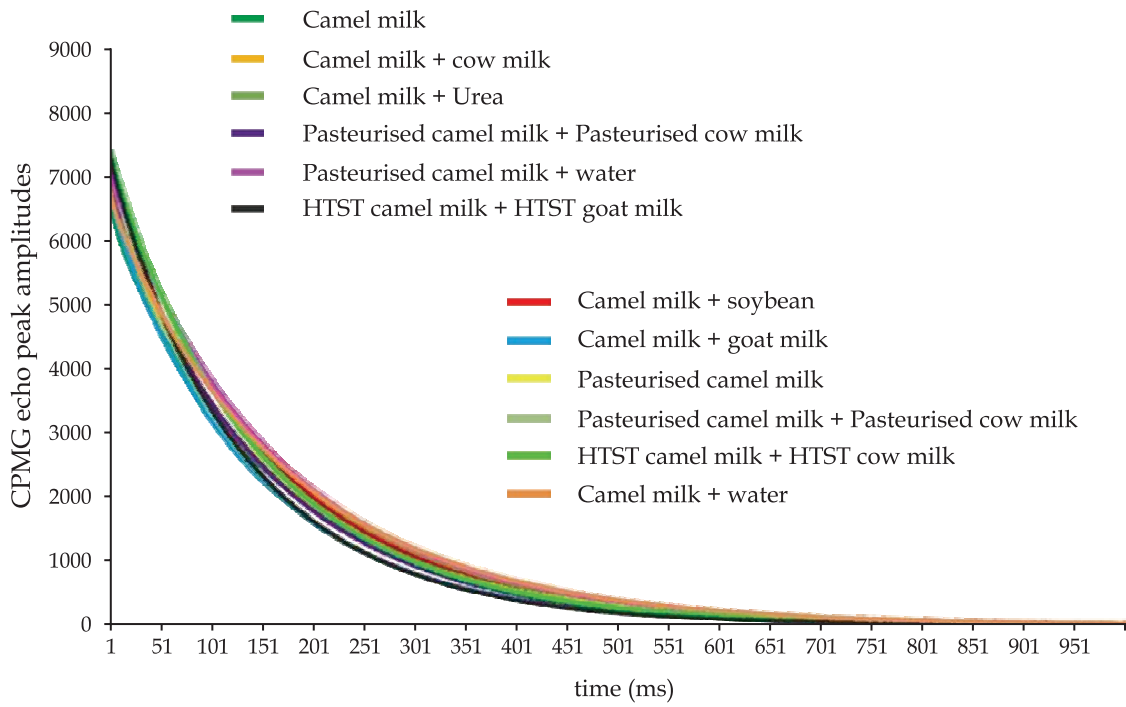

Fig 2. CPMG echo peak amplitudes of Alashan adulterated camel milk.

Results and analysis of adding urea to camel milk

Adding urea to Xinjiang camel milk

The pc1 and pc2 retained 99.93% of the original data on the score map (Fig 17) for Xinjiang camel milk samples adulterated with urea. As the proportion of urea added increased, the distribution of the samples shifted from the top to the bottom of the pc2 axis. The distributions of pure camel milk and mixed samples containing 4% urea (2 mL) did not overlap, which showed that urea can be detected. Table 6 shows that the T_2 values of the mixed samples gradually increased with the increasing proportion of urea added.

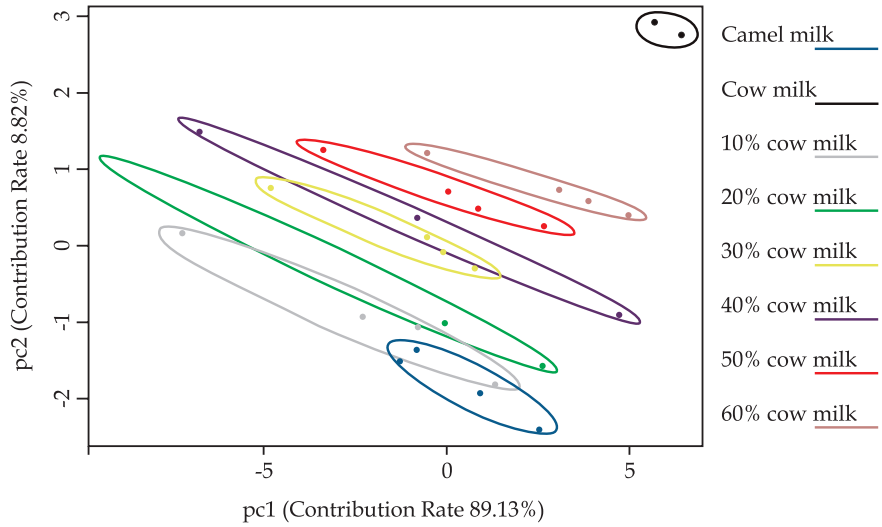

Fig 3. PCA scores of Xinjiang pure camel milk and different adulterated milk samples.

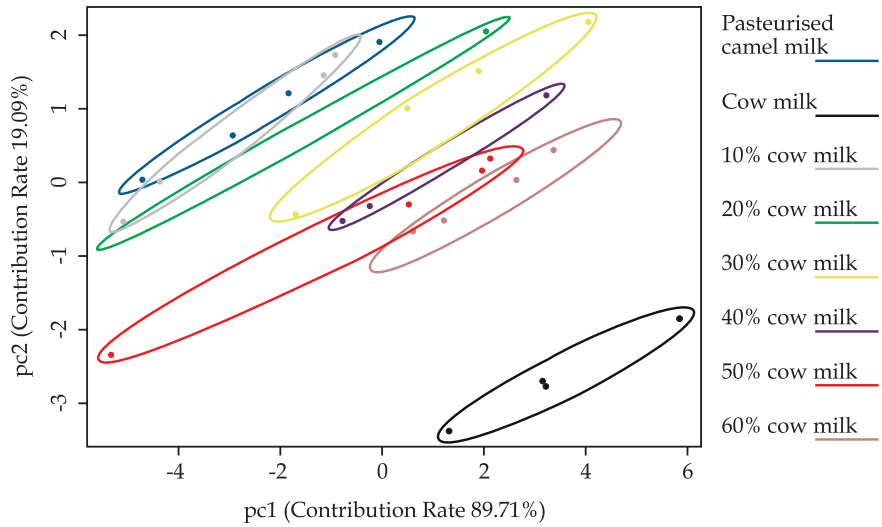

Fig 4. PCA scores of Xinjiang pasteurised camel milk and different adulterated milk samples.

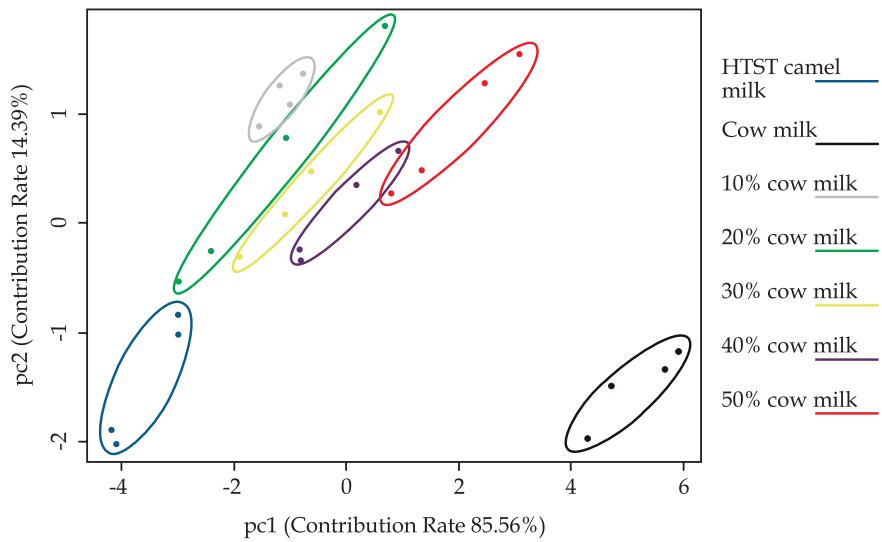

Fig 5. PCA scores of Xinjiang HTST camel milk and different adulterated milk samples.

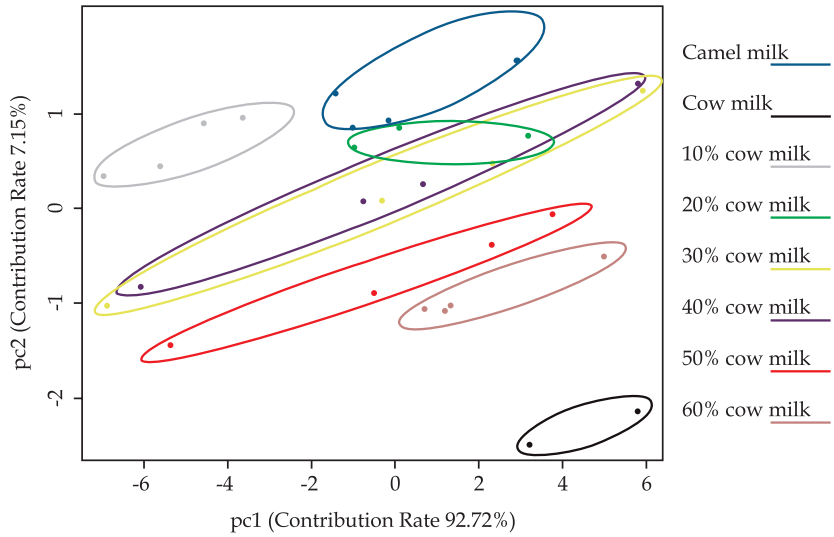

Fig 6. PCA scores of Alashan pure camel milk and different adulterated milk samples.

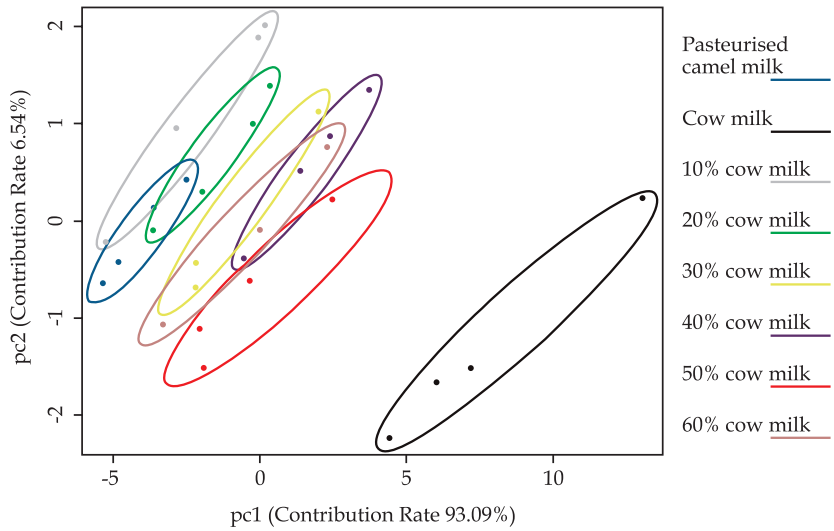

Fig 7. PCA scores of Alashan pasteurised camel milk and different adulterated milk samples.

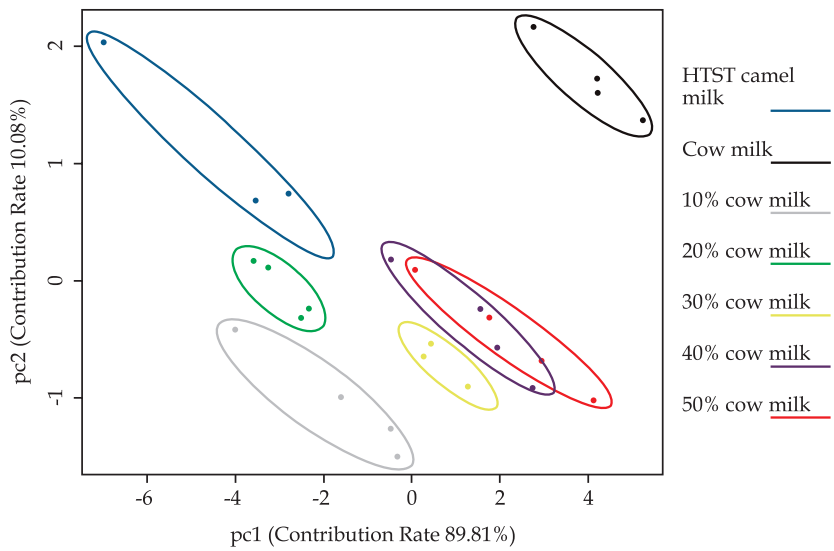

Fig 8. PCA scores of Alashan HTST camel milk and different adulterated milk samples.

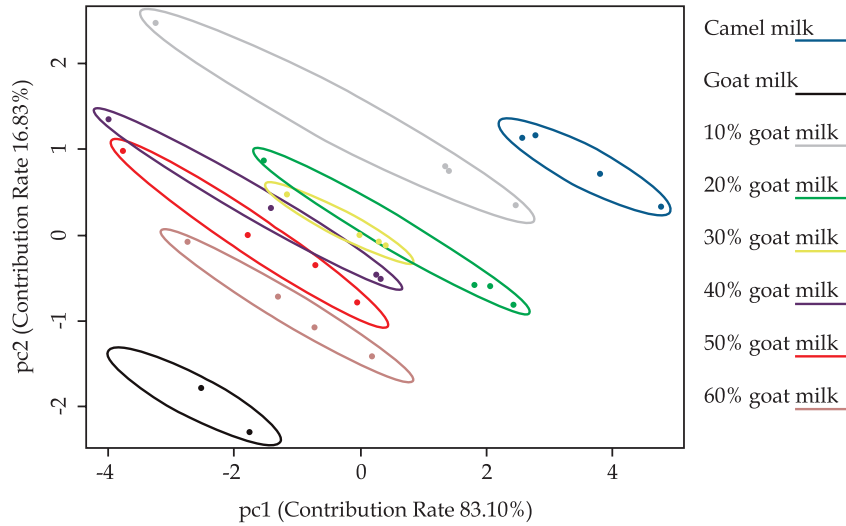

Fig 9. PCA scores of Xinjiang pure camel milk and different adulterated goat milk samples.

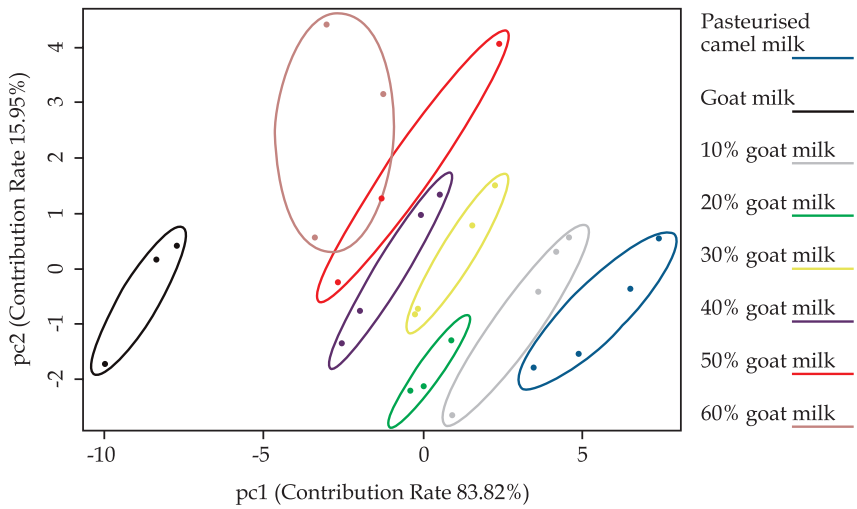

Fig 10. PCA scores of Xinjiang Pasteurised camel milk and different adulterated goat milk samples.

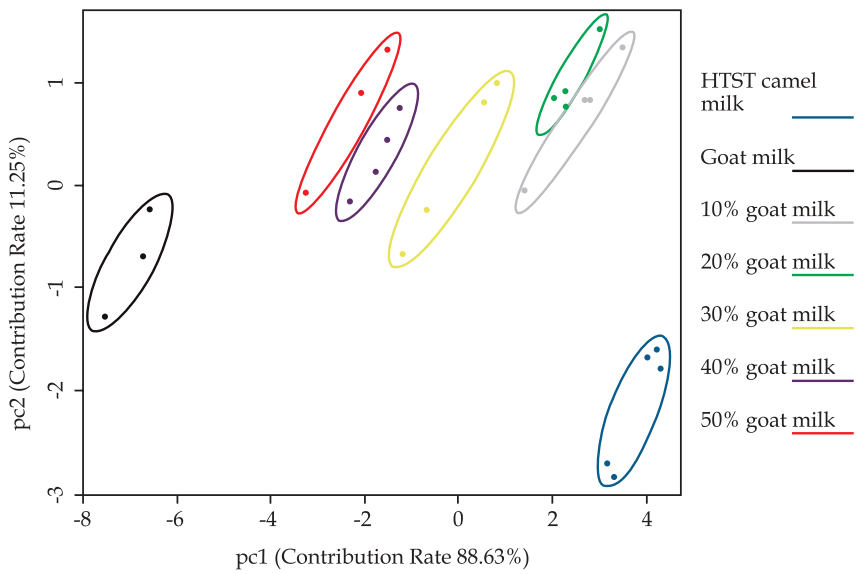

Fig 11. PCA scores of Xinjiang HTST camel milk and different adulterated goat milk samples.

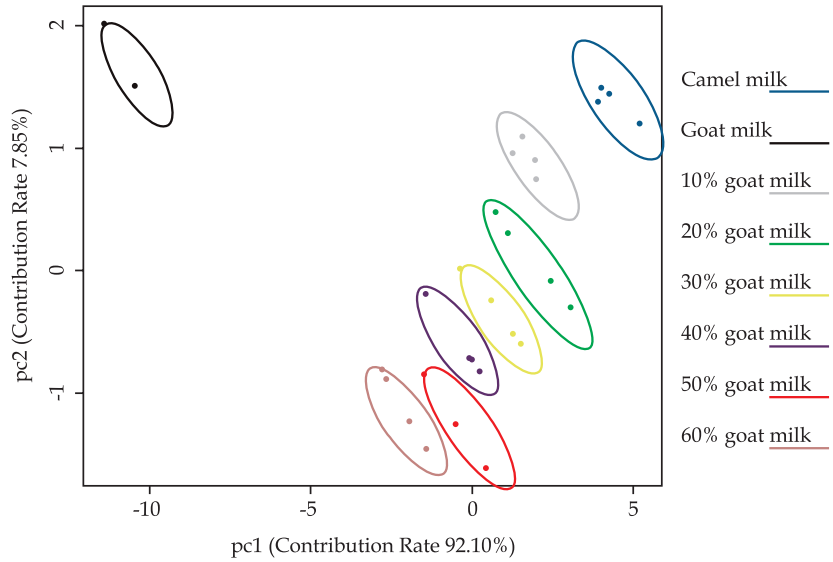

Fig 12. PCA scores of Alashan pure camel milk and different adulterated milk samples.

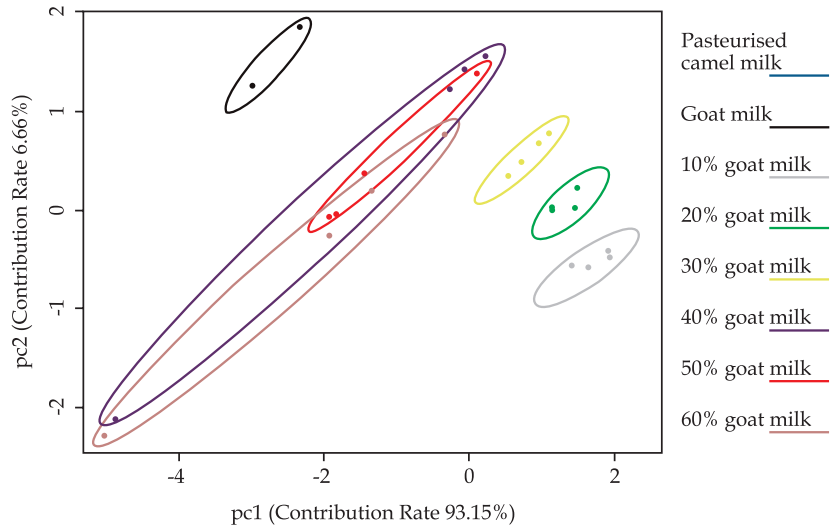

Fig 13. PCA scores of Alashan pasteurised camel milk and different adulterated goat milk samples.

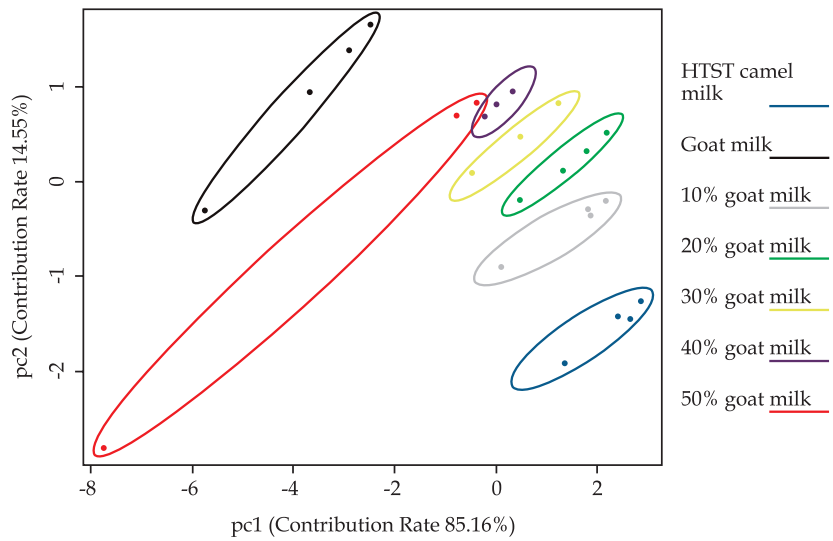

Fig 14. PCA scores of Alashan HTST camel milk and different adulterated goat milk samples.

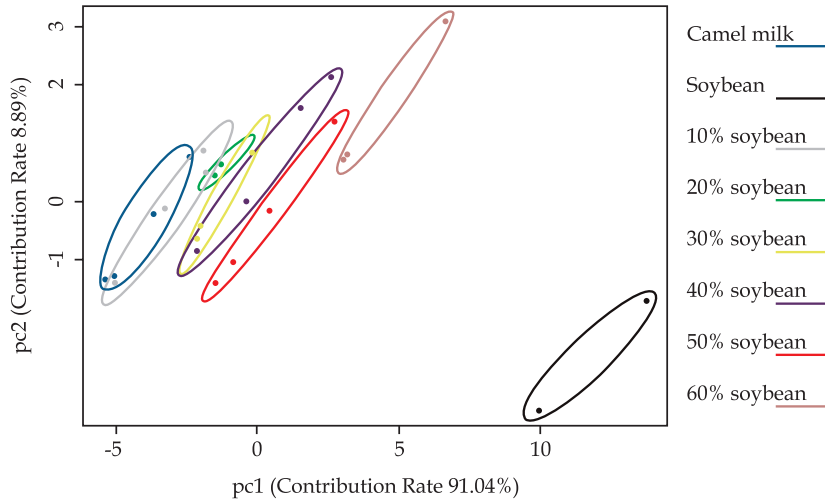

Fig 15. PCA scores of Xinjiang raw camel milk and different adulterated soybean milk samples.

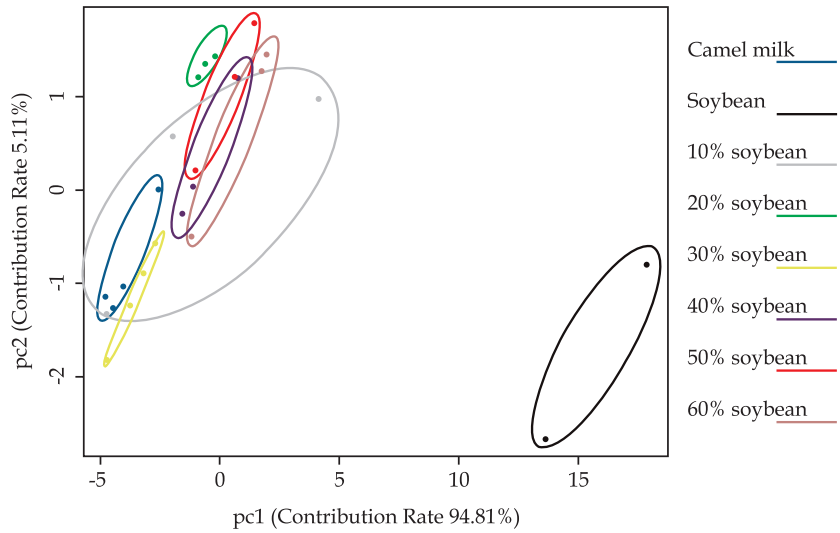

Fig 16. PCA scores of Alashan raw camel milk and different adulterated soybean milk samples.

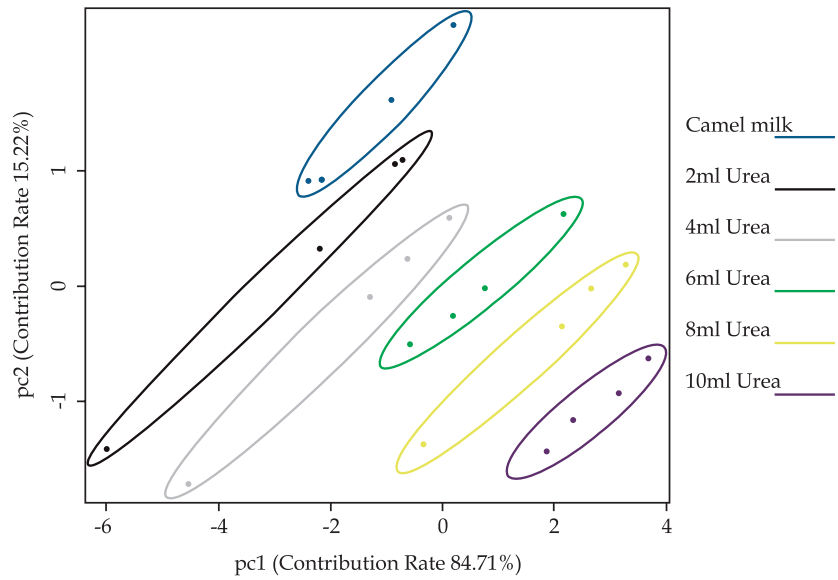

Fig 17. PCA scores of Xinjiang raw camel milk and different adulterated urea samples.

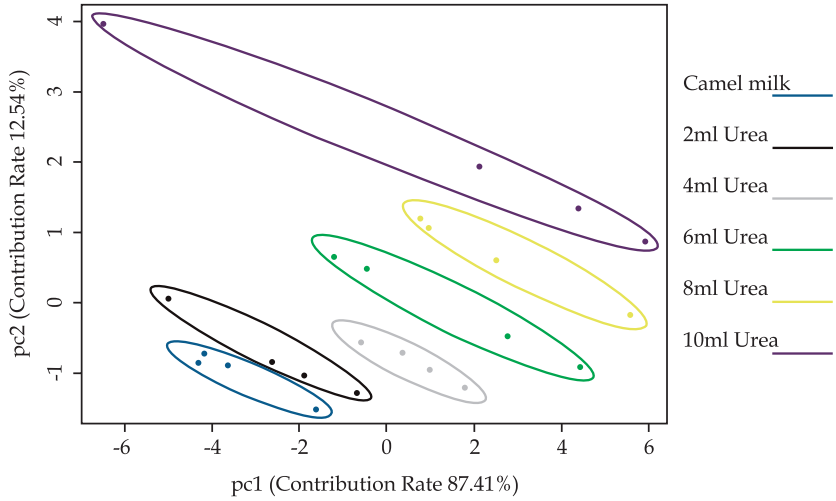

Fig 18. PCA scores of Alashan raw camel milk and different adulterated urea samples.

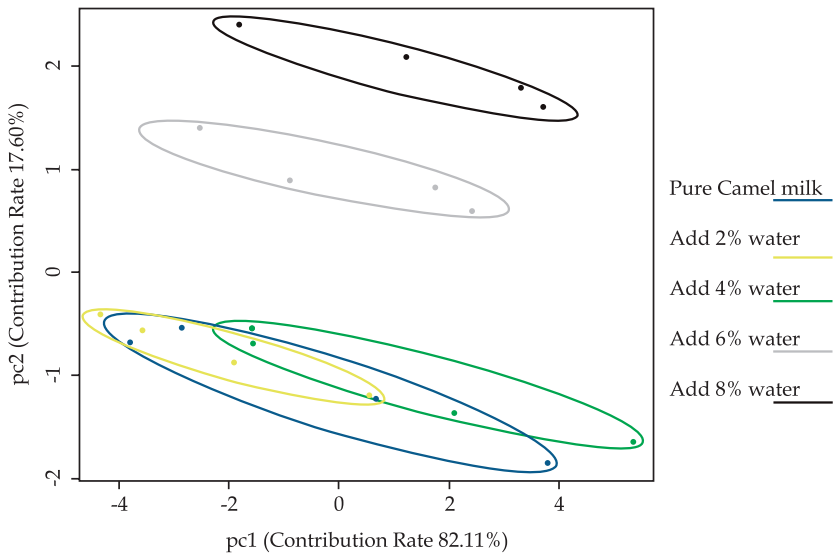

Fig 19. PCA scores of water detection limit in Xinjiang adulterated camel milk.

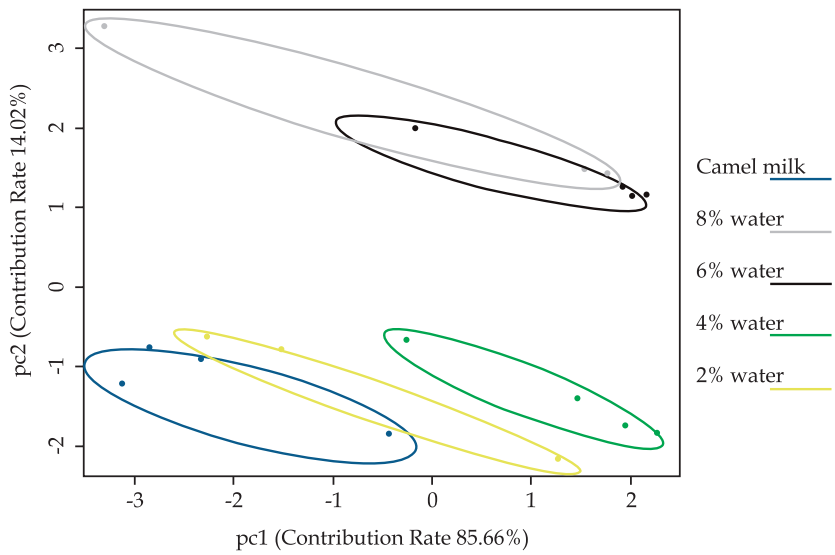

Fig 20. PCA scores of water detection limit in Alashan adulterated camel milk.

Adding urea to Alashan camel milk

The pc1 and pc2 retained 99.95% of the original data, as shown in (Fig 7) for Alashan camel milk samples adulterated with urea. As the proportion of urea added increased, the distribution of the samples shifted from the bottom to the top of the pc2 axis. The detection results were the same as those for pure Xinjiang camel milk (Table 6).

Results and analysis of adding water to camel milk

Adding water to Xinjiang camel milk

The addition of water to raw milk is common in milk adulteration because it is the cheapest adulteration method. Water-adulterated Xinjiang camel milk samples underwent low-field NMR detection, which the results showing that samples were well differentiated on the pc axes and that pc1 and pc2 retained 99.71% of the original data. As the proportion of water added increased, the distribution of samples shifted from the bottom to the top of the pc2 axis. As shown in Fig 19, the lower limit of the water content was between 2 and 4%. Meanwhile, the T_2 values of the mixed samples gradually increased with the increasing proportion of water added (Table 7).

Adding water to Alashan camel milk

The Alashan camel milk samples adulterated with water were well differentiated on the pc- axis, with pc1 and pc2 retaining 99.68% of the original data. As the proportion of water added increased, the distribution of samples shifted from the bottom to the top of the pc2 axis. As shown in Fig 20 and Table 18, the lower limit of the water content was 2%.

The low-field NMR techniques combined with PCA successfully detected regular changes in all samples from different regions on the PCA axes. These results were consistent with previous results for the low-field NMR detection of adulterated milk (Jiang and Han, 2010).

In this study, the sample distributions on the PCA axes were similar to those of adulterated milk reported by Jiang and Han (2010) which shows that the application of NMR techniques to the identification of adulterated camel milk is feasible.

Our results for adulterated camel milks from different regions showed that the T_2 values of low-field NMR detection for raw, pasteurised and HTST-treated camel milks from the same area were not much different.

Furthermore, the results showed that the T_2 values of adulterated camel milks from Xinjiang and Alashan were quite different, which might be due to the feeding management, grazing environment and drinking water of camels in the different regions.

This PCA analysis showed that the total contributions of pc1 and pc2 retained over 99% of the raw data of adulterated camel milk.

However, as the proportion of the adulterated substances was relatively high, it is not known whether the low-field NMR analysis can detect smaller adulterated concentrations. Therefore, for applications of low-field NMR to the identification of adulterated camel milk, it is necessary to determine the lower limit of detection of common adulterated substances.

In this study, the detection limit of the camel milk samples adulterated with cow milk was higher than that of other adulterated samples, mainly due to the similar presence and distribution of water in

Table 5. T_2 values of Xinjiang raw camel milk samples adulterated with soybean milk.

Camel milk Sample	10% soybean	20% soybean	30% soybean	40% soybean	50% soybean	60% soybean	Soybean
79.33±0.31	80.53±0.75	83.12±0.35	85.25±0.15	87.11±0.72	91.24±0.08	95.31±0.12	151.78±0.09
96.61±0.67	97.73±0.6	99.57±0.12	102.89±0.23	106.34±0.34	110.66±0.18	119.24±0.45	151.78±0.09

Table 6. T_2 values of Xinjiang raw camel milk (XRCM) and Alashan raw camel milk (ARCM) samples adulterated with urea.

Camel milk Sample	4% Urea	8% Urea	12% Urea	16% Urea	20% Urea
79.33±0.31 (XRCM)	80.23±0.49	81.66±0.26	83.78±0.34	85.9±0.07	88.32±0.19
96.61±0.67 (ARCM)	97.53±0.58	98.44±0.49	101.06±0.39	103.68±0.3	105.30±0.21

Table 7. T_2 values of water detection limit in Xinjiang adulterated camel milk (XRCM) and Alashan raw camel milk (ARCM).

Camel milk Sample	2% water	4% water	6% water	8% water
79.33±0.31 (XRCM)	80.21±0.08	80.90±0.12	84.10±0.16	86.46±0.19
96.61±0.67 (ARCM)	97.71±0.21	99.95±0.05	104.36±0.23	105.80±0.12

the camel milk. Generally, the viscosity of camel milk depends on the camel variety, feeding conditions and drinking water. Herein, by combining low-field NMR techniques with PCA analysis, adulterated milks from different regions have been successfully detected. The detection of other adulterants that might be mixed into camel milk requires further investigation.

Low field NMR is an effective method for the rapid detection of adulterated camel milk. The data obtained showed that: (i) The detection limits were ≥10% (≤20%) for pure and pasteurised Xinjiang milk adulterated with cow milk, ≤10% for HTST-treated adulterated Xinjiang camel milk, ≤20% for pasteurised Alashan milk adulterated with cow milk and ≤10% for pure and HTST-treated adulterated Alashan camel milk; (ii) the results for pure, pasteurised and HTST-treated Xinjiang and Alashan camel milks adulterated with goat milk were essentially the same as for those adulterated with cow milk, with a mixing ratio reaching ≤10% being fully detected; (iii) the detection limit for Xinjiang and Alashan camel milks adulterated with soybean milk was ≥10% (≤20%); (iv) the detection limits of Xinjiang and Alashan camel milks adulterated with urea were ≤4%; and (v) the detection limit of Xinjiang camel milk adulterated with water was ≥4% (≤6%), while that of Alashan camel milk adulterated with water was ≥2% (≤4%).

Acknowledgements

This work was supported by grants from the Major Special Project of the Inner Mongolia Autonomous Region. We would like to thank the native English speaking scientists of Elixigen Company (Huntington Beach, CA, USA) for editing our manuscript.

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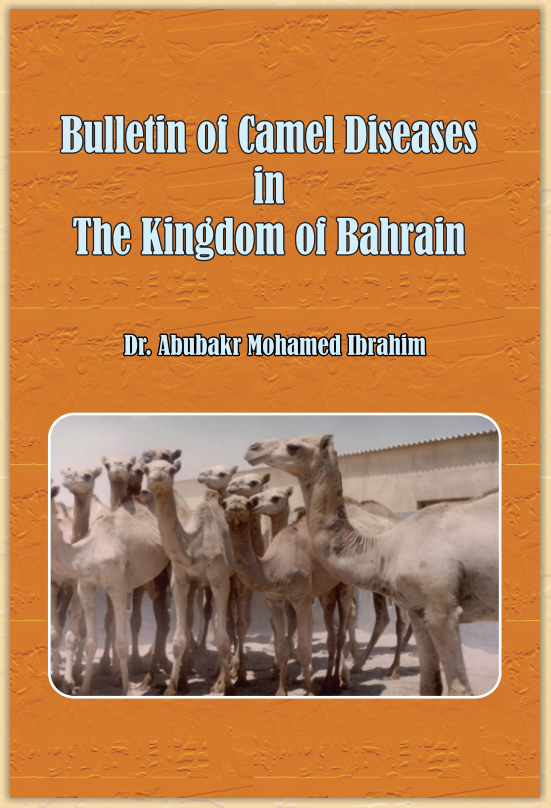
Bulletin of Camel Diseases in The Kingdom of Bahrain

This is a unique book which contains chapters on infectious and non-infectious diseases. The chapter on infectious diseases contains six sections. The section of bacterial diseases is subclassified as corynebacterium abscesses, paratuberculosis, hepatic necrobacillosis, mastitis, *Streptococcus zooepidemicus,* bacterial Infection in young camels, uterine Infection, infection of the vagina and vulva and other disorders. The section of protozoal diseases has narrations on trypanosomiasis, anaplasmosis and babesiosis. The section on parasitic infections is composed of gastrointestinal parasites in young camels, echinococcosis and mange. The section of mycotic diseases contains phycomycosis and ringworm. The section of viral diseases contains subsections on camel pox and contagious ecthyma. Edema Disease is described in miscellaneous section. The chapter on noninfectious diseases has three sections. Other section on poisoning describes pyrethroid, nitrate and toxic jaundice. The section describes zinc deficiency. The miscellaneous section describes foreign bodies, sand colic, bloat, caecal impaction, hydrocephalus, corneal opacity and osteochondroma.

About the Author

Dr. Abubakr Mohamed Ibrahim is a Veterinary Pathologist and worked for a long period as head of Royal Court Veterinary Laboratory. Kingdom of Bahrain which led to genesis of this publication out of his rich experience in diagnosing camel diseases in the Kingdom of Bahrain. This would be counted as his significant contribution and future researchers will find it easy to understand the pattern of camel diseases in this part of the world. Dr. Abubakr had majority of his publications based on camel diseases of Bahrain. Thus publication of this book would prove an important reference book for the camel practitioners and researchers.



Editor:
Dr. T.K. Gahlot

Edition: 2014

Publisher:
Camel Publishing House
67, Gandhi Nagar West,
Near Lalgarh Palace,
Bikaner-334001 Rajasthan, India
email: tkcamelvet@yahoo.com

Website:
www.camelsandcamelids.com
www.tkgahlotcamelvet.com

Price: US $ 90
INR 1000

ISBN 81-903140-2-5

DOI : 10.5958/2277-8934.2018.00039.5 Vol 25 No 3, p 289-290

DROMEDARY MASTITIS CAUSED BY *Clostridium septicum:* A CASE REPORT

J. Kinne, E. Maio, M. Joseph, S. Anas and U. Wernery
Central Veterinary Research Laboratory, P.O. Box 597, Dubai, United Arab Emirates

A dromedary camel in lactation which previously had delivered a healthy baby, died suddenly. It showed classical lesions caused by *Clostridium septicum* including oedematous mastitis.

Clostridium (C.) septicum causes malignant oedema, which is an acute, generally fatal toxaemia affecting all species and ages of animals. The disease occurs worldwide and is often found in connection with other anaerobes like *C. chauvoei, C. novyi, C. perfringens* and *C. sordellii. C. septicum* is found in soil and intestinal contents of animals.

Predisposing factors like wounds, parturition, shearing, docking, castration may activate the dormant spores and subsequently disease may develop within 6 to 12 hours as fast replication of the pathogen occurs releasing its exotoxins. These toxins cause local and systemic pathological signs which include inflammation, oedema, necrosis and gangrene. *C. septicum* can also cause braxy in sheep, a highly fatal infection characterised by toxaemia and inflammation of the abomasal wall. Literature on malignant oedema in Old World Camels (OWC) is scarce. However, malignant oedema is an economically important disease in alpacas in Peru and has also been associated with rattle snake bites in llamas in Colorado (Wernery *et al,* 2014).

Fig 1. Oedematous udder in lactation caused by *C. septicum.*

We describe here a *C. septicum* mastitis followed by sudden death of a lactating dromedary.

Materials and Methods and Results

An adult dromedary carcass with a history of sudden death was submitted for necropsy to CVRL. It showed massive swelling of the udder (Fig 1). It was treated with antibiotics but died 6 hours later.

Autopsy

Autopsy of the female camel in good condition (714 kg) revealed swollen haemorrhagic left quarters and right back quarter and pale right front quarter. The mammary lymph nodes were enlarged, with the left mammary lymph node showing haemorrhages. Subacute suppurative to necrotising mastitis with lot of bacteria was observed in histology.

Udder tissues from all 4 quarters, mammary lymph nodes, lung, liver and intestines were examined bacteriologically using routine methods including anaerobic incubation of blood agar-based Zeissler agar with selective supplement (SR0093E, Oxoid) for 18 hours at 37˚C. Heavy growth of *C. septicum* was found in all 4 mammary gland tissues, udder lymphnodes, liver and lung. Additionally, *C. perfringens* was isolated from the small intestines in high numbers and confirmed as an alpha toxin producer by an ELISA from BIO X Diagnostics, Belgium. *C. septicum* showed a typical growth pattern on Zeissler agar with swarming, beta haemolysis and stringent odour. Subculture were made and identification were carried out by Gram stain and using Vitek ANC anaerobe cards (Biomérieux 21347). However, it should be mentioned that differentiation between *C. septicum* and *C. chauvoei* is difficult. Toxins from both are antigenically identical (El Idrissi and Ward, 2010).

Discussion

C. septicum may penetrate the body through wounds and scratches contaminated with soil or through the digestive tract mucosa. Anaerobes are

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common inhabitants of the camel's environment and their intestines. The bacilli replicate when stimulating conditions occur releasing potent necrotoxins as well as exotoxins causing oedema and necroses. The present case was a good milking camel which recently delivered a healthy calf which could have contributed to its death caused by *C. septicum*.

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DOI : 10.5958/2277-8934.2018.00040.1 Vol 25 No 3, p 291-294

EFFICIENCY OF SINGLE-DOSE ADMINISTRATION THERAPIES FOR TREATMENT OF ENDOMETRITIS IN THE CAMELS (*Camelus dromedarius*)

I.M. Ghoneim[1, 2], W. El-Deeb[1,3] and H.F. Al-Fehaed[4]

[1]Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, PO Box 1757, Al Ahsa 31982,
[2]Department of Theriogenology, College of Veterinary Medicine, Cairo University, Giza 12211,
[3]Department of Veterinary Medicine, Infectious Disease and Fish Diseases,
Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt
[4]Ministry of Agriculture, Kingdom of Saudi Arabia

ABSTRACT

The present study was performed to compare the efficacy of different treatment regimes for clinical endometritis in dromedary camels. The animals were randomly distributed into 6 treatment groups. Camels in group I (n=20) were treated with intrauterine injection of 20 g of MastiVeyxym®. In group II (n=15), III (n=14), IV (n=14) and V (n=19) the animals were treated with intrauterine wash with 300 ml of 0.1 % acriflavine. Besides, intrauterine injection with Metricure®, intramuscular injection with 500 µg Estrumate®, intramuscular injection with 20 mg oestradiol and intrauterine administration of 3 g of chlortetracycline in groups II, III IV and V respectively. The animals (n=14) in group VI receiving sexual rest of one follicular cycle. Once a camel had been tried to be treated, the animal was serviced in the second estrus after the treatment. The conception results were 40%, 60%, 42.86%, 42.29%, 42.11% and 21.4% in group I, II, III, IV, V and VI, respectively. The highest (P <0.05) efficiency of the treatment was recorded in-group I (uterine wash with 0.1 % acriflavine besides intrauterine injection with Metricure®).

Key words: Acriflavine, camel, endometritis, fertility

Reproductive disorders cause infertility leading to a tremendous impact on the economical profit of animal production (Bellows *et al*, 2002; Inchaisri *et al,* 2010). Numerous uterine disorders have been recorded in camelids and possibly play a paramount role in reduced fertility in these species (Tibary and Anouaassi, 1997; Khalafalla *et al,* 2017). Uterine infections are regarded to be the most common cause of reproductive deficiency in camels (Wernery, 1991; Tibary and Anouassi, 2001; Tibary, 2004; Ali *et al,* 2010b; Nabih and Osman, 2012; Mshelia *et al,* 2014). The main reasons were overbreeding, postpartum complications and unhygienic gynaecological handling (Tibary, 2004). Contrary to other domestic species, there are limited data on treatment of uterine infections in camels (Tibary *et al,* 2006). Application of policies for the diagnosis and treatment of endometritis in the aspect reported for other farm animals are common in camel practice (Tibary and Anouassi, 2001). There is dearth in the studies evaluating the efficiency of different therapies of endometritis in camelids. The objective of the present study was to assess the efficiency of single administration of 5 therapies for camel endometritis on the subsequent fertility.

Materials and methods

Animals

The study was performed on 121 repeat breeding dromedary camels admitted to the Veterinary Teaching Hospital (during one year). Camels had a history of failing to conceive after more than two services. Animals aged between 6-20 years and 2 experienced a wide range of management systems that varied with regard to housing, feeding and aspects of breeding. Clinical endometritis was diagnosed in 96 female camels based on history, rectal and vaginal examination as well as by trans-rectal ultrasound using linear-array 5 MHz transducer (UST-588U-5, SSD-500V, ALOKA, Co., Japan).

Treatment protocols

Camels suffering from endometritis were randomly distributed into 6 treatment groups. Group I (n=20) received an intrauterine injection of 20 g of Masti Veyxym® (Veyx Pharma Schwazenborn,

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Germany), having the enzymes chymotrypsin (16mg), trypsin (16mg) and papain (8 mg), 200,000 IU of retinolpalmitat and 240 mg of α-tocopherol acetate. The uteri of the animals in groups II (n=15), III (n=14), IV (n=14) and V (n=19) were washed with 300 ml of 0.1% acriflavin (C14N14CIN3, Flukachemie EG, Buchs, Switzerland). In addition, animals in group II, III, IV and V received intrauterine injection of Metricure®. (Schering-Plough Animal Health Ltd), intramuscular injection of 500 µg Estrumate® (Interrvet Schering-Plough Animal Health), intramuscular injection of 20 mg oestradiol benzoate (Intervet-Boxmeer Holland) and intrauterine injection of 3 g of chlortetracycline (Vetoquinol S.A.), respectively. Animals in group VI received sexual rest of one follicular cycle.

Assessment of treatment efficiency

After treatment, the female dromedaries were serviced in the 2nd oestrus post-treatment. The reproductive activity was monitored at next 3 months after treatment and conception rate of all groups was recorded.

Statistical analysis

All data were analysed using Chi-square analysis (SPSS 16.0 statistical software, 2007). Values were considered significant at P<0.05.

Results and Discussion

Table 1 represents the number of the female dromedaries treated by different therapies and the animals conceived after treatment. In group I, II, III, IV and V, 8 animals out of 20 (40%), 9 animals out of 15 (60%), 6 animals out of 14 (42.86%), 6 animals out of 14 (42.86 %) and 8 animals out of 19 (42.11%) were conceived, respectively. In non-treated group, 3 animals out of 14 (21.4%) were conceived. Group II had a significantly higher conception rate (P <0.05) than groups I, III, IV and V, which had no significant differences among each other. A significantly high (P <0.05) efficiency of the treatment was recorded in group II.

The study revealed that the percentage of the conceived animals in group I was 40%. Most antibiotics and chemicals adversely interfere with the uterine defence mechanism (Vandeplassche, 1981). Proteolytic enzymes have been recorded in the non-antibiotic therapy of mastitis in dairy cattle (Krüger *et al,* 1999). The use of proteolytic enzymes as an intrauterine treatment of endometritis in cows was also reported (Drillich *et al,* 2005). Protease inhibitors potently suppress the growth of a variety of

Table 1. Conception rate in female camels after treatment by five different treatment protocols and control.

Group	Treatment	Total treated	Number conceived (%)
I	MastiVeyxym®	20	8 (40)[a]
II	Acriflavin + Metricure®	15	9 (60)[b]
III	Acriflavin + Estrumate®	14	6 (42.8)[a]
IV	Acriflavin + oestradiol benzoate	14	6 (42.8)[a]
V	Acriflavin + chlortetracycline	19	8 (42.1)[a]
VI	Non treatment group	14	3 (21.4)[c]

Columns with different superscript differ significantly (P < 0.05).

pathogenic bacterial and fungal strains (Rakashanda *et al,* 2012). The effects of chymotrypsin on bacterial growth commonly depended on the strains of bacteria and the concentrations of chymotrypsin. Papain is a plant proteolytic enzyme for the cysteine proteinase family. The enzyme is able to do lysis of organic molecules made of amino acids in bacteria (Amri and Mamboya, 2012). Vitamin E increases the uterine health (Arrehiga *et al,* 1994) and decrease the incidence of metritis (Erskine *et al,* 1997). Acriflavin is known to inhibit mitochondriogenesis (Hill and Anderson, 1969). Using acriflavin solution, Ali *et al* (2010 b) reported 64% conception rate in dromedary camel suffering from endometritis. In this study, the highest (60%) conception rate after the treatment was recorded in group II. Cephapirin the active principle present in Metricure® has a broad spectrum of activity against gram-positive and gram-negative bacteria. (Donowitz and Mandell, 1988). Cephapirin is resistant to the action of penicillinase and is active in an anaerobic environment such as encountered in a septic uterus (Adams, 2001). Parallel to our results, Swelum and Alowaimer (2013) reported 76.2% conception rate after treatment with cephapirin in dromedary camels. Intrauterine infusion of cephapirin improved reproductive performance of cows with clinical endometritis (LeBlanc *et al,* 2002) and subclinical endometritis (Kasimanickama *et al,* 2005; Ahmadi *et al,* 2005; Wagener *et al,* 2017). In group III the conception rate was 42.8%. Exogenous $PGF_{2\alpha}$ may augment immune functions or stimulate the uterine motility to aid the uterus to resolve the infections (Hirsbrunner *et al*, 2003; Nakao *et al,* 1997). In cattle, the success rate of $PGF_{2\alpha}$, or its analogues, for the treatment of endometritis ranged from 49% - 92% (Jackson, 1977; Coulson, 1978; Steffan *et al,* 1984; Murray *et al,* 1990 and Chaffaux *et al,* 1991).

However, the role of $PGF_{2\alpha}$ in eliminating uterine infections is not known (Lewis, 2003). The conception rate was 42.8% in Group IV. Oestrogen stimulates the uterine tone to aid in evacuating abnormal uterine content (Bretzlaff and Ott, 1981) as well as, increase the production of mucous that contains host defense compounds and induce the oestrus, which markedly activate neutrophil phagocytosis and resistance of the uterus to infection (Smith and Risco, 2002).Treatment of female camels in group V resulted in conception rate of 42.1%. Several authors (Gustafsson, 1984; Montes and Pugh, 1993; Sheldon and Noakes, 1998) endorsed the use of oxytetracycline for intrauterine infusion. Oxytetracycline fulfils most of the parameters for indicating an antimicrobial substance for the treatment of endometritis (Masera *et al,* 1980; Bretzlaff *et al,* 1983; Noakes, 1991; Makki *et al,* 2017). In the current study, 21.4% (n=14) of the animals received only sexual rest conceived. The camels exposed to oestradiol for relatively long time during the follicular phase (Manjunatha *et al,* 2012; Musa and Abusineina, 1978). Consequently, the endometrial blood supply and uterine defense mechanism increase (Liu and Hansen, 1993; Youngquist and Shore, 1997; Noakes *et al,* 2002) which may be a cause for spontaneous recovery. We conclude that cephapirin is the drug of choice for treatment endometritis in female dromedary camel.

Acknowledgements

The authors acknowledge the Deanship of Scientific Research at King Faisal University for their support under grant No. 17122001.

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DOI : 10.5958/2277-8934.2018.00041.3 Vol 25 No 3, p 295-302

HEPATIC CYSTIC ECHINOCOCCOSIS IN CAMELS OF SAUDI ARABIA: PREVALENCE, RISK FACTORS AND ECONOMIC LOSS

F.A. Al-Hizab[1], M.A. Hamouda[1], O.H. Amer[2], A.M. Edris[3], A.M. Ibrahim[1,4], S.M. Abdel-Raheem[3,5] and W.R. El-Ghareeb[3,6]

[1]Department of Pathology, [2]Department of Clinical Laboratory Science, College of Applied Medical Sciences, University of Hail, Saudi Arabia

[3]Department of Veterinary Public Health and Animal Husbandry, College of Veterinary Medicine, King Faisal University, Saudi Arabia

[4]Department of Pathology, College of Veterinary Medicine, Suez Canal University, Egypt

[5]Department of animal nutrition and clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Egypt

[6]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt

ABSTRACT

The study was conducted to determine the prevalence, risk factors and economic loss of hepatic cystic echinococcosis (CE) in the dromedary camel in Eastern Region, Saudi Arabia. A total number of 810 animals were selected randomly out of 3400 (23.8%) dromedary camels and subjected to careful ante-mortem inspection. The examined animals were 330, 270 and 210 from Al Omran, Al Ahsa and Al Dammam abattoirs, respectively. Out of 810 slaughtered dromedary camels, 216 were found infected with hydatid cyst (26.7%) with an occurrence rate 27.6%, 29.6% and 21.4% from Al Omran, Al Ahsa and Al Dammam abattoirs, respectively. Statistically, the rate of infection was not significantly different among the three examined abattoirs (Chi-square 4.29, P >0.05). Majaheem breed was more liable to be infected and had higher infection prevalence (29.6%) in comparison with Magateer (22.8%) and Wadha (10.3%) breed (OR 0.65; P <0.01). Female camel had higher infection rate (33.4%) than male (16.3%) (OR 2.58; P <0.001). The probability of infection with CE linearly increased with the camel age and the probability increased by 2.09 fold for each further year of age (OR 2.09; P <0.001). Camel with poor body condition was significantly more likely to be infected (29.7%) in comparison with camel with medium (26.1%) and well body condition (9.3%) (OR 0.69; P <0.01). The recorded cysts were also examined for fertility and viability. The percentages of fertile and viable cysts found to be higher in Al Omran region 4/91 (4.4%), followed by Al Ahsa, 2/80 (2.5%) and Al Dammam, 1/45 (2.2%), respectively. The overall direct financial loss in three abattoirs per year was 304,092 $. Hepatic cystic echinococcosis is highly prevalent in one humped camel causing considerable economic loss. Further studies are needed to estimate indirect economic loss and determine the genotypes of such disease in KSA.

Key words: Cystic echinococcosis, economic loss, prevalence, risk factors

The prevalence of cystic echinococcosis (CE) is high in South America, Australia and North Africa (McManus and Thompson, 2003). It is endemic in the countries of the Mediterranean Sea and Arabian Peninsula. Several previous studies documented the endemic and zoonotic properties of cystic echinococcosis in various provinces of Saudi Arabia (Fadaladdin *et al,* 2013; Toulah *et al,* 2017). According to the Annual reports of Ministry of Health, Saudi Arabia (2006-2012), the prevalence of human cystic echinococcosis in Saudi Arabia is low ″about ten cases per year″, however its prevalence in the livestock animals is considerable. Diagnosis of hydatid disease in living animals is very difficult with no routine reliable test. Detection of cysts during post-mortem examination is considered the most consistent diagnostic tool (OIE, 2008). The risk factors controlling the prevalence of hydatidosis are connected with existing specific social, cultural, environmental and epidemiological situations. However, dogs and human behaviours play significant roles in the epidemiology and the dynamic of the disease (Ochi *et al,* 2015). WHO reports consider hydatid disease as one of the neglected diseases (WHO, 2017). However, the disease remains to be a considerable cause of morbidity and mortality in many countries and had a strong economic loss include retardation of growth, condemnation of the whole carcass or infected viscera and reduces the

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quantity and quality of meat. The global economic losses attributed to infection of domestic animals with CE were over 2 billion dollars in 2006 (Budke *et al,* 2006). Therefore, it required strict control measures that may take around 20 years of sustained efforts to eliminate such disease (Craig *et al,* 2007).

There is a lack of information about the prevalence, epidemiology and economic losses caused by cystic echinococcosis in most Gulf countries particularly in Saudi Arabia. Therefore, these were included in objectives of the present study which were done on Saudi dromedary camels.

Materials and Methods

Study design and sample size

The sample size was designed using the formula: $N= (Z^2xP) (1-P)/e^2$ as described by Dohoo *et al* (2010), where the N = Total number of sample size, Z=1.96 for 95% confidence interval, e=5% desired absolute precision and P=15% expected true prevalence. Based on the previous criteria, 197 samples per locality (abattoir) were required to get an accurate estimation of the prevalence. Hence, 330, 270 and 210 animals were examined from Al Omran, Al Ahsa and Al Dammam abattoirs, respectively between December 2016 to February 2018. The samples were collected with the intention of maximising the sample size to increase precision. The whole number of slaughtered animals during the phase of this study was approximately 1600, 1000, 800 in Al Omran, Al Ahsa and Al Dammam abattoirs respectively. The sampling interval was computed based on the study period, the whole number of animals slaughtered as well as the required sample size. Therefore, the sampling interval was 5 (1600/330) in an Al Omran abattoir, 4 (1000/270) in Al Ahsa abattoir and 4 weeks (800/210) in Al Dammam abattoir (Thrusfield, 2005).

Abattoir survey

In the current study, a total number of 810 animals out of 3400 (23.8%) dromedary camels were selected randomly and subjected to careful ante-mortem inspection to be examined for the detection of cystic echinococcosis in the study areas. Breed, age, sex and body condition of each animal was noted. Local camel breeds were Majaheem, Magateer and Wadha. The age of the animals was < 5 year, 5-10 year and > 10 year based on the dentition formula (Khan *et al,* 2003). Scoring of the body condition was performed according to the guidelines prepared by Faye *et al* (2001) which was based on the condition of the back and flank as follows: poor (1), medium (2) and well (3).

Post mortem inspection

Visceral organs of slaughtered camels were subjected to a thorough visual inspection, palpation and systematic incision with special attention to the liver. The total number of cysts of each infested liver was counted and recorded. Cyst was dissected out and was separately collected in clean container. These were subsequently subjected to a systematic size measurement using Varnier caliper and classified as small cyst (< 4cm), medium cyst (4-8cm) and a large cyst (> 8cm) (Schantz, 1990).

Parasitological examination

Cysts was carefully incised and its contents were evacuated into a sterile test tube for microscopic examination of protoscolices.

A. Examination of cysts fertility

The cysts were classified into fertile and infertile based on the presence of either free protoscileces or brood capsules. Further, the infertile cysts were subsequently categorised as sterile (fluid filled cysts), or calcified cysts have a gritty sound sensation upon incision (Assefa *et al,* 2015).

B. Examination of cyst viability

Following the characterisation of cysts as fertile, viability tests were applied to figure out whether they were alive or dead. To confirm the viability of cyst, one drop of hydatid fluid was mixed with one drop of aqueous solution 0.2% eosin (W/V) and then examined microscopically (40×) (Dalimi *et al,* 2002). If the protoscolex was unable to gain the stain, it was considered live, whereas the dead one is stained uniformly.

Histopathological examination

Collected specimens were fixed using 10% neutral formalin. Five micron paraffin sections were stained with hematoxylin and eosin (H&E).

Estimation of direct loss

According to Romazanov (1983), the direct financial loss due to hepatic hydatidosis was assessed by the following formulae: Loss due to condemned infected livers = Number of livers condemned per year × Average weight of the liver × Price of the liver per kg. Number of livers condemned per year were taken from annual record of each abattoir.

Statistical analysis

The obtained data were evaluated and analysed via SPSS 16.0 for Window's software. The prevalence

of CE was calculated as the per cent of infected animals. Chi-square ($\chi 2$) as a statistical test was performed to determine the association between the various risk factors like different abattoir, sex, age, body condition, breed and the prevalence of (CE) in the examined camels. To predict the probability of hepatic (CE) in slaughtered camels in the eastern region of KSA when breed, host sex, age and body condition was used as a risk factors, logistic regression was carried out with each variable fitted separately. The odds ratio (OR) and 95% confidence interval (CI) were estimated for all variables. A statistically significant association among variables considered to exist (P value < 0.05).

Results

The hepatic CE were diagnosed grossly as multiple greyish white nodules with a thick wall and cavity. Their diameter ranged from millimetres to centimetres in size (Fig 1A). In some cases, the cavity contained a viscid material or was calcified. The cyst was also diagnosed histologically by the presence of three layers, adventitia (pericyst), laminated membrane (ectocyst) and germinal epithelium (endocyst) (Fig 1B). Finally the cysts were diagnosed by a parasitological examination (Fig 1C, D).

Prevalence of hepatic cysts and risk factors:

The overall prevalence rate of hepatic CE was 26.7% (216/810). The prevalence at Al Omran, Al Ahsa abattoirs and Al Dammam was 27.6, 29.6 and 21.4%, respectively. The rate of infection was not significantly different among camel slaughtered in the three examined abattoir in the Eastern region of KSA (Chi-square 4.29, P > 0.05) (Table 1).

The results displayed in table 2 and 3 indicated that the prevalence of CE was significantly associated with camel breed (Chi square 8.34, P < 0.05). Majaheem breed was more liable to be infected and had higher infection prevalence (29.6%) in comparison with Magateer (22.8%) and Wadha (10.3%) breed. These results were confirmed by the logistic regression analysis which revealed that with each unit increase in breed categories from 1 to 3 (1, Majaheem; 2, Magateer; 3, Wadha), the likelihood of infection with CE was decreased (OR 0.65; P <0.01; at 95% CI 0.48-0.88). Hence, Majaheem breed was more likely to acquire the infection with CE higher than Magateer and Wadha breeds, respectively.

A significant positive association was noticed in the current study between camel sex and the probability of infection with CE (Chi square 28.92, P<0.001). Female camel had higher infection prevalence (33.4%) than male (16.3%). The probability of female camels to be infected was higher by 2.58 fold than male (logistic regression analysis: OR 2.58; P <0.001; at 95% CI, 1.81-3.66).

A significant association was found between camel age and likelihood of infection with CE in all examined camel carcasses (Chi square 30.36., P<0.001). The probability of infection with CE linearly increased with the camel age and the probability increased by 2.09 fold for each further year of age (logistic regression analysis: OR 2.09; P <0.001; at 95% CI, 1.6-2.73).

Table 1. The overall prevalence (%) of cystic echinococcosis in camels in Eastern region of KSA.

City	Abattoir	No.* examined	Infected		Chi-square (χ^2)	P value
			No	Prevalence		
Al-Ahsa	Al-Omran	330	91	27.6	4.29	0.12
	Al-Ahsa	270	80	29.6		
Al-Dammam	Al-Dammam	210	45	21.4		
Total		810	216	26.7		

*No. = Number, χ^2, Chi- square at (P < 0.05) considered significant.

Table 2. The logistic regression predicting the probability of hepatic cystic echinococcosis in camels in Eastern region of Saudi Arabia.

Variable	Coefficient	S.E.	DF	P value	OR	95% C.I	
						Lower	Upper
Breed	- 0.43	0.15	1	<0.01	0.65	0.48	0.88
Sex	0.94	0.18	1	<0.001	2.58	1.81	3.66
Age	0.74	0.14	1	<0.001	2.09	1.6	2.73
Body condition	-0.36	0.13	1	<0.01	0.69	0.53	0.9

OR, Odds ratio, DF= degree of freedom, CI= confidence interval

A significant association was present between camel body condition and the likelihood of infection with CE (Chi square 10.26, P<0.01). The likelihood of infection with CE was decreased by 69% (logistic regression analysis: OR 0.69; P <0.01, at 95% CI, 0.53-0.9) each unit increase in body condition (1 poor, 2 medium, 3 well). Therefore, camel with poor body condition were more likely to be infected (29.7%) in comparison with camel with medium (26.1%) and well body condition (9.3%).

Cyst Characterisation, fertility and viability

The number of cysts ranged from only one detectable cyst to numerous cysts that were distributed throughout the liver lobes without any obvious predilection site. Cyst's size was 1-2 cm in diameter and reached up to 3 cm (<4cm). Some cysts had thin, translucent wall and contained clear watery fluid. The fertile cysts were examined directly without stains (wet mount) (Fig 2A, B, C). Living cysts refused eosin stain (Fig 3A) and were stained with special stain (Fast green) (Fig 3B). Dead cysts get eosin stain (Fig 3C). The occurrence of fertile, strile and calcified cysts is given in Table 4.

Direct loss of cystic echinococcosis

A number of condemned infected livers/ year was 3022, 2592, 937 at Al-Omran, Al-Ahsa and Al-Dammam abattoirs, respectively. The overall direct financial loss in three abattoirs per year = 140.279+120.318+43.495=304,092 $ (Fig 4).

Discussion

In the present study, varous tests used to detect HCE were reliable diagnostic tools (Bacciarini *et al,* 2004; Bowman, 2009). The prevalence of CE was 26.7%, which is in agreement with the reports of previous studies in Al- Madinah and Al Baha cities (34.64% and 32.85%). On the other hand, the prevalence in Mecca and Jeddah cities was 16% and 6.86%, respectively. This variation could be ascribed to the existence of some stray dogs and wild carnivores, which are

Table 3. Risk factors of cystic echinococcosis in camel slaughtered in 3 abattoirs in Eastern region in KSA.

Risk factors	No. examined	No. infected	Prevalence %	Chi-square (χ^2)	P value
Breed				8.34	<0.05
Majaheem	513	152	29.6		
Magateer	268	61	22.8		
Wadha	29	3	10.3		
Total	810	216	26.7		
Sex				28.92	<0.001
Male	319	52	16.3		
Female	491	164	33.4		
Total	810	216	26.7		
Age					
< 5 years	81	7	8.6	30.36	<0.001
5-10 years	329	71	21.6		
> 10 years	400	138	34.5		
Total	810	216	26.7		
Body Condition				10.26	<0.001
Poor	384	114	29.7		
Medium	372	97	26.1		
Well	54	5	9.3		
Total	810	216	26.7		

Table 4. Characterisation of cystic echinococcosiss in three abattoirs.

City	Abattoir	Total	Fertile				Sterile		Calcified	
			Viable No.	(%)	Non-viable No	(%)	No.	(%)	No.	(%)
Al-Ahsa	**Al Omran**	**91**	**4**	**4.4**	**1**	**1.1**	**23**	**25.3**	**63**	**69.2**
	AL Ahsa	**80**	**2**	**2.5**	**0**	**0**	**33**	**41.2**	**45**	**56.3**
AlDammam	**AlDammam**	**45**	**1**	**2.2**	**0**	**0**	**5**	**11.1**	**39**	**86.7**
Total	**All abattoir**	**216**	**7**	**3.24**	**1**	**0.46**	**61**	**28.24**	**147**	**68.06**

*No. = Number

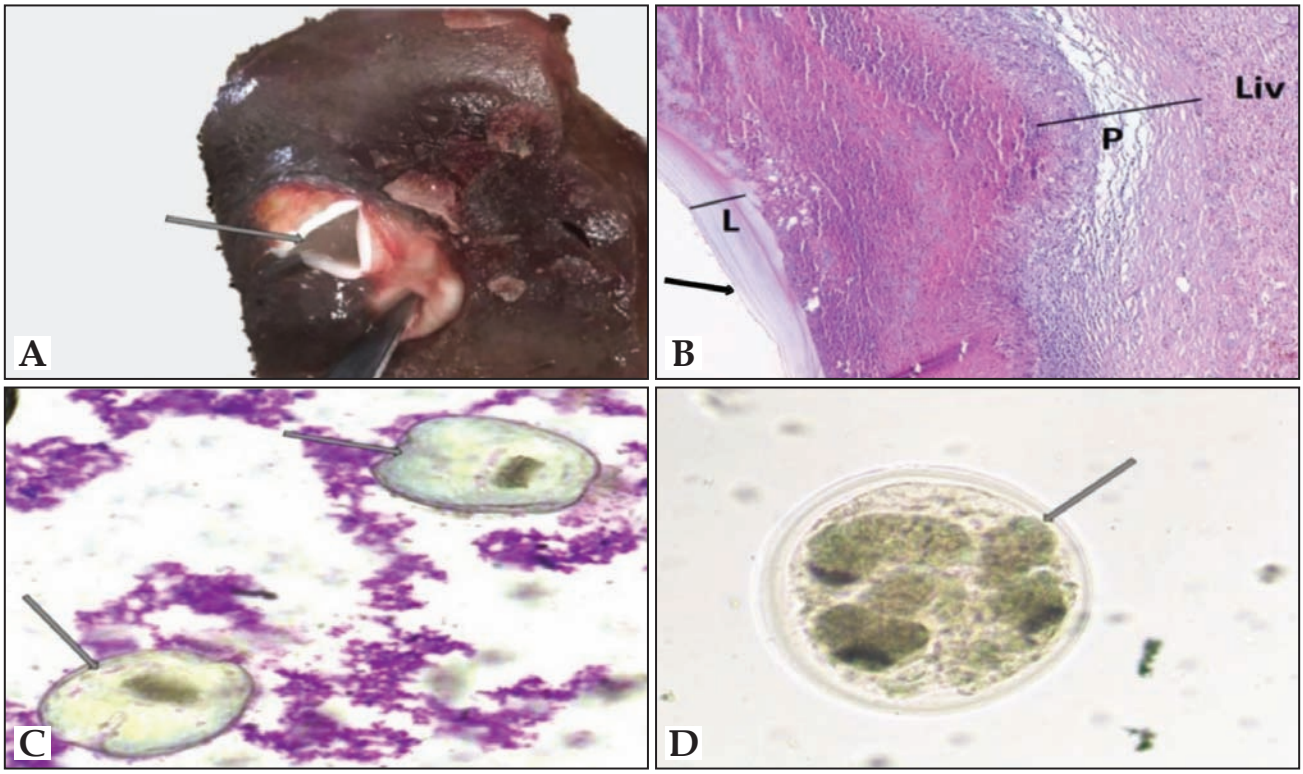

Fig 1. A, grossly large cyst (arrow) is seen embedded in the hepatic tissue containing germinal layer. B, histopathologically. Hepatic tissue (Liv) is effaced with the cyst that is formed of three layers germinal layer (arrow), laminated layer (L), pericyst layer (P, 10X). C, wet mount shows live invaginated protoscolices which did not stain with 0.1% eosin (arrows). D, wet mount displays fast green stained viable cyst (arrow).

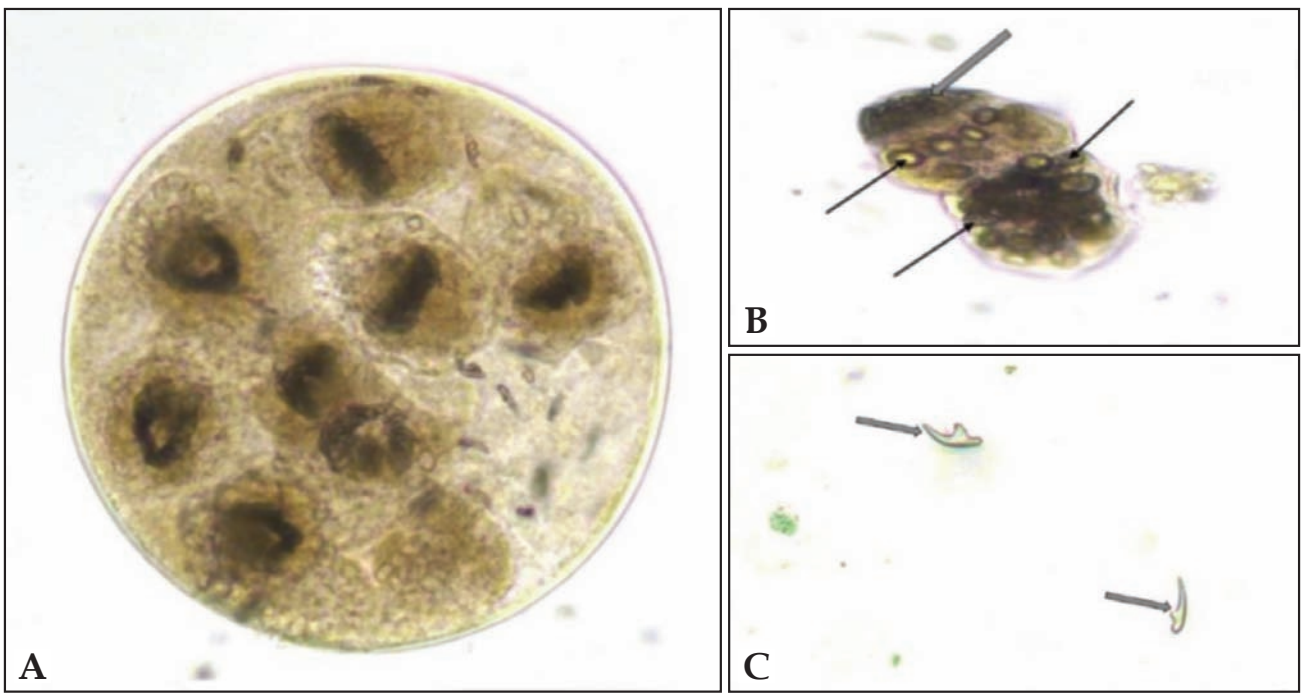

Fig 2. Wet mount - unstained cystic echinococcosis: A, Invaginated Scolices. B, Evaginated Scolices with double row rostellar hooklets (Thick arrow) and calcareous corpuscles (Thin arrows). C, Note the hooks, Large hooks: 25.0 (µm) Small hooks: 17.5 (µm).

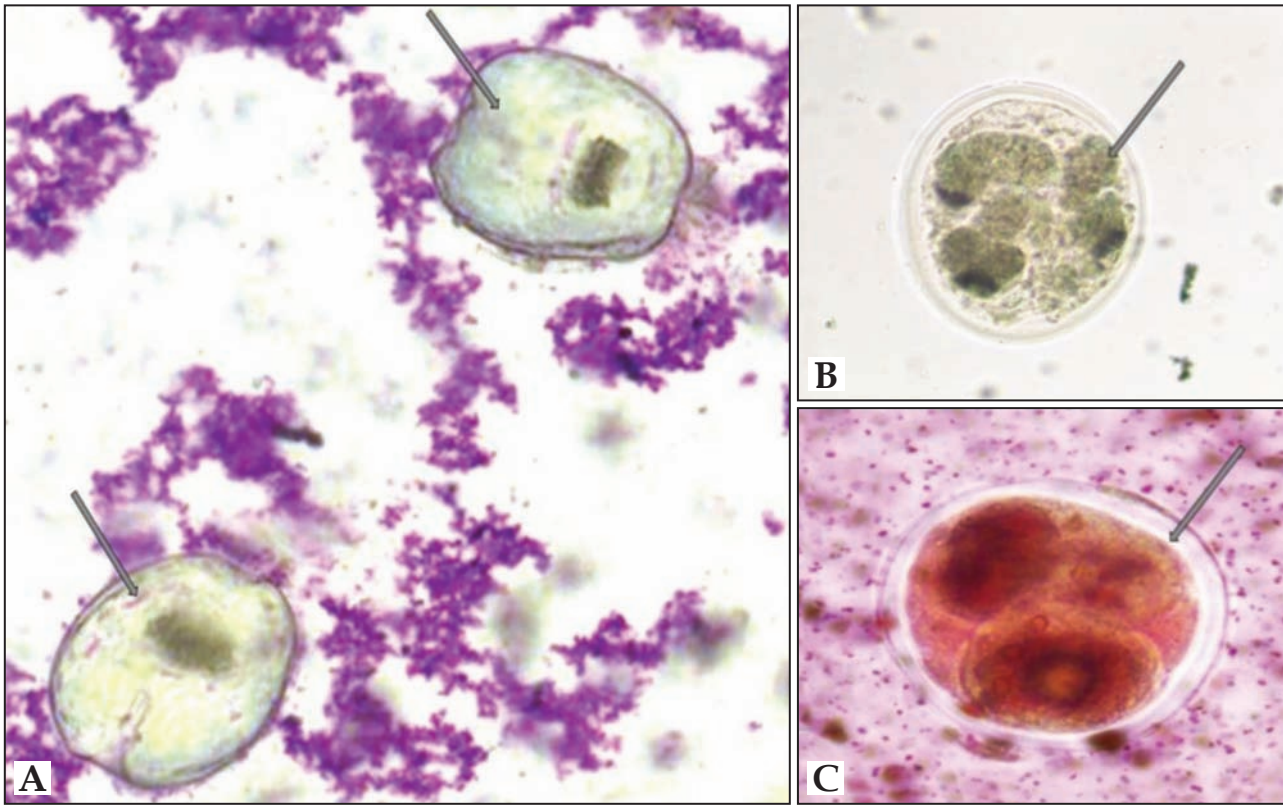

Fig 3. Wet mount - stained cystic echinococcosis: A, live invaginated protoscolices refuse staining with 0.1% eosin (arrows). B, Fast green stained viable cyst (arrow). C, Dead invaginated protoscolices staining with 0.1% eosin (arrow).

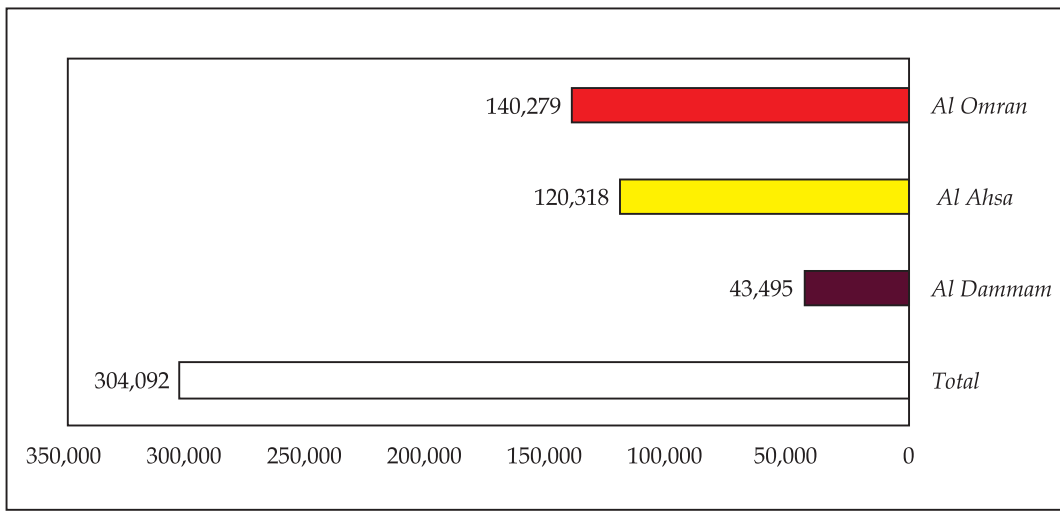

Fig 4. Annual financial loss ($) due to *E. granulosus*.

the major factor for life cycle of *E. granulosus*. The wide increase in offering the uncooked offals to pet animals around the home, lack of correct treatment for adult *E. granulosus* in dogs, inadequate public awareness about the disease, lack of good fencing of abattoirs, lack of accurate meat inspection measures in the abattoir, the bad tradition of disposing dead wild or domestic animals unburied and left open for scavenging carnivores produce suitable conditions for the spreading of the disease (Akeberegn *et al,* 2017). Previous surveys indicated significant variations among species in CE prevalence, however, studies differed on which species is most highly affected. Some studies reported higher prevalence in small ruminants compared to other species (Toulah *et al,* 2017), whereas other studies, observed a higher prevalence in camels (Fadaladdin *et al,* 2013). The prevalence rate of CE was 39.6% and 5.3% in camel

in Kuwait and the Sultanate of Oman respectively (Abdul-Salam and Farah, 1988; Al Kitani *et al,* 2015). The wide range differences in the prevalence of CE in the various countries and regions of the country could be due to the differences in livestock health management and related risk factors among the societies of the study areas.

Majaheem breed was more liable to be infected and had higher prevalence in comparison with Magateer and Wadha breed because Majaheem breed is kept for milk production and the most common breed in the Eastern region and usually present in central Saudi Arabia, with some spread eastward (Mehaia *et al,* 1995). Wadah Breed are present mainly in western and northen part of the country more than the Eastern region (Mehaia *et al,* 1995).There was a significant association between the prevalence of CE and camel sex as a risk factor. Females were more probable to have CE infection than male camels as previously reported by Ibrahim (2010) in cattle, sheep and camels. Females are more vulnerable to the infection by metacestode of *E. granulosus*, than the males. Females stay longer for reproductive purposes than males, so the probability of having the infection is higher than in males. This result was in agreement with the findings of Abdul-Salam and Farah (1988) from Kuwait, Gizachew *et al* (2013) from Ethiopia. Similarly, old age camels had a risk for infecion than younger animals due to long exposure of old age camels to infective stage of *E. granulosus* with lower immunity against the infection. In our study, the infection prevalence was higher in the older age classes. The association between age and the infection rate among camels in harmony with results of previous studies (Rinaldi *et al,* 2008; Ibrahim, 2010; El-Ghareeb *et al,* 2017).

In this study, animals with poor body condition were likely to be highly infected with CE. This could indicate that the affected animals have decreased productivity (wool and milk), lowered fertility, decreased hide value and retard growth (Torgerson *et al,* 2003; Battelli, 2009). The fertility of cysts is an important factor that can influence the life cycle of a disease. The study showed that 3.4% of the cysts were fertile, 34.03% was sterile and 62.6% was calcified. The fertility rate is lower than those observed in camels (51.57%) and sheep (18.18%) (Moghaddas *et al,* 2014). This variation could be attributed to the geographical situation, the nature of infected hosts, the sites of infection and genotype dependant (McManus, 2006). The high proportion of sterile and calcified cysts in camel may generally imply that most of the cysts in camel are infertile and this underscores the role of camel in maintaining the life cycle of a disease. In addition, a high number of calcified cysts in the liver may be due to abundant fibrous tissue and reticuloendothelial cells (Haftu and Kebede, 2014).

In this study, the financial loss due to CE was confined to condemnation of affected livers, which estimated 304,092 dollars, annually in Eastern region, Saudi Arabia. This may reflect the erratic veterinary and extension delivery services to key stakeholders in the country. Moreover, the global economic losses attributed to infection of domestic animals with CE were over 2 billion dollars in 2006 (Budke *et al,* 2006). In Jordan total livestock-associated costs due to CE were approximately 3.6 million dollars (Torgerson *et al*, 2001), while it costs 51,900 dollars annually in Iran (Daryani *et al,* 2007). Despite the low prevalence and low attention of CE in human beings, it is still considered a life threatening zoonotic disease with a high public health importance (Craig *et al,* 2007).

In conclusion, hepatic cystic echinococcosis considered one of the most important parasitic diseases with economic implications in camels in the eastern Region. Consequently, an effort should be made to control the transmission of CE from abattoirs by the safe disposal of infected offal, control of stray dogs, creation and spreading of public awareness about the zoonotic importance of hydatidosis. Further studies are needed to estimate indirect economic loss (body weight, decreased hide value and milk yield) and determine the genotypes of CE in camel in Saudi Arabia.

Acknowledgement

The authors would like to acknowledge the financial support provided by King Abdulaziz City for Science and Technology (KACST) through project no. (Arp-35-169), Saudi Arabia. Authors also thank Dr. Mohammed Mokbel for his assistance during samples collection.

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DOI : 10.5958/2277-8934.2018.00042.5 Vol 25 No 3, p 303-306

URINALYSIS OF PREGNANT AND NON-PREGNANT ALPACAS (*Vicugna pacos*) AND BACTRIAN CAMELS (*Camelus bactrianus*)

A. Kubátová and T. Fedorova
Department of Animal Science and Food Processing, Faculty of Tropical Agri Sciences,
Czech University of Life Sciences Prague, Kamýcká 129, Praha - Suchdol, 165 00 Czech Republic

ABSTRACT

The aim of this study was to test the possibility of using urinalysis in health control or alternatively for pregnancy diagnosis in non-invasively obtained samples from female alpacas and Bactrian camels kept in central Europe. Urine samples were collected from 12 female alpacas from three farms and from 14 female Bactrian camels from four zoos in the Czech and Slovak Republics. Samples were collected repeatedly at intervals of 4–9 weeks from 2010 to 2014. Spontaneous urination of animals was used to collect fresh urine samples into 0.5 L plastic cups held by hand or fastened to a telescopic rod. Immediately after sampling, the samples were tested using Duotest® double zone pH-indicator papers and DekaPhan® Leuco diagnostic test strips to obtain information about the specific gravity, the pH and the presence of leucocytes, nitrites, proteins, glucose, ketones, urobilinogen, bilirubin, blood and haemoglobin. In camels, urine colour was also observed. There were no problems with urine collections in the majority of animals thus non-invasive urine sampling was concluded as useful in camelids. However, none of the measured parameters showed a difference between pregnant and non-pregnant females ($p > 0.05$). The obtained results can serve as control values for urinalyses performed in camelids kept in small farms and zoos in the central Europe.

Key words: Alpacas, bactrian camel, urine, urinalysis

Alpacas and camels are important domestic animals in tropical and subtropical regions (Djemali and Alhadrami, 1998; Vilá Melo and Gutiérrez Vásquez, 2012), but they are also commonly kept as domestic or zoo-housed animals and pets (Gillespie and Flanders, 2010; Fowler, 2010). Urine testing can be useful not only for pregnancy diagnosis but also for health status monitoring (Czekala *et al,* 1990; Ganswindt *et al,* 2002; Rodríguez *et al,* 2017), because urinary pH can be used for the diagnosis of nutritional disorders (Nappert and Naylor, 2001) or post parturient diseases (Markusfeld, 1987). Urinary pH of healthy camelids ranges between 7.0 and 8.5; specific gravity from 1.018 to 1.050 (Cebra *et al,* 2013). According to a study by Banerjee *et al* (1981) urine of pregnant camels is darker, more alkaline (8.3 ±0.25 *vs.* 7.4 ± 0.37) and its specific gravity is higher than in non-pregnant female camels (1.086 ± 0.003 *vs.* 1.036 ± 0.01).

However, regardless of usefulness of urinalysis, laboratory methods are either unavailable or too expensive in some areas (Lanari *et al,* 2007), thus the data from urinalysis of camelids kept in small farms and zoological gardens of temperate regions are very limited or missing.

The aim of the present study was to test the possibility of using accessible diagnostic test strips intended for simple human urinalysis for basic non-invasive health control in female alpacas bred on small farms and zoo bred female Bactrian camels in two steps: 1) to compare the indicative results of urinalysis obtained *via* test strips designed for human use with the results obtained *via* more precise indicator papers; 2) to compare the indicative results of urinalysis with the health status of animals at the time of sampling. Additionally, the possibility to use basic urinalysis as a simple pregnancy diagnostic method in camelids was tested.

Materials and Methods

Urine samples were collected from both pregnant and non-pregnant 12 female alpacas (*Vicugna pacos*) and 14 female Bactrian camels (*Camelus bactrianus*) kept in 3 private farms and 4 zoos (Bratislava, Plzeň, Prague and Ústí nad Labem), respectively, in the Czech and Slovak Republics. In pregnant females, the urine was collected in different phases of pregnancy period. For each pregnancy, the date of parturition was recorded so the length of pregnancy could be calculated. In 1 alpaca female,

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abortion occurred during the research period so the date of parturition was estimated by a veterinarian according to the development of the foetus. Non-pregnant females were sampled repeatedly during different months of the year in the same time as pregnant animals.

In all alpacas, feeding was based on a combination of hay and pasture *ad libitum*, supplemented by recommended dosage of concentrated feed for alpacas. Camels were fed mainly by meadow hay *ad libitum*, supplemented by vegetables and pellets for herbivores, or cracked oats; fresh fodder was sometimes provided during the spring time or animals had access to grassy pasture. Fresh water was provided *ad libitum* in all breeding facilities.

Samples were repeatedly collected at intervals of 4–9 weeks during April 2013 – February 2014 for alpacas and September 2010 – November 2011 for camels. Urine samples were collected during spontaneous urination of animals throughout the whole day using 0.5 L plastic cups held in the hand or fastened to a telescopic rod (Fedorova *et al,* 2015).

Immediately after sampling, the pH was tested using Duotest® double zone pH-indicator papers (Macherey-Nagel GmbH and Co. KG, Germany). The specific gravity, pH and the presence of leucocytes, nitrites, proteins, glucose, ketones, urobilinogen, bilirubin, blood and haemoglobin was tested using DekaPhan® Leuco diagnostic test strips for urinalysis (Erba Lachema s.r.o., Czech Republic). Measurements obtained by DekaPhan® were considered approximate, because these test strips were designed for human use. In camels, urine colour, density and temperature were also evaluated, measured using a glass hydrometer and thermometer, respectively. The specific gravity was calculated from the urine density.

The data were analysed using the Statistica Cz 12 program (StatSoft, Inc., 2013).

Results and Discussion

There were no problems with urine collections in the majority of animals. Three alpacas (25%) and three camels (21%) were a little bit timid and urine collection was more complicated than in others. Total 60 samples from alpacas (31 from non-pregnant, 29 from pregnant females) and 62 from camels (21 from non-pregnant, 41 from pregnant females) were collected. The basic urine parameters measured in tested animals are shown in table 1. No significant difference was found ($p > 0.05$) between the pH as measured by DekaPhan® and Duotest® in either species, so we can conclude that it is adequate to use DekaPhan® for indicative measurement of pH values even though DekaPhan® is less precise than Duotest® due its less specific scale. All samples were within the interval considered normal for ruminant urinary pH (Sundra *et al,* 2004; Salles *et al,* 2012), with one single exception where a pH 5.5 sample was collected from a camel affected by diarrhoea.

Table 1. Basic urine parameters measured in female alpacas and Bactrian camels.

		Alpacas (n = 60)			Camels (n = 62)		
		Value	Min	Max	Value	Min	Max
Hydrometer	density [kg/m^3][a]	-	-	-	1,033.00 ± 2.60	1,010.00	1,080.00
	specific gravity[a]	-	-	-	1.03 ± 0.00	1.01	1.08
Duotest®	pH[a]	8.33 ± 0.06	7.00	8.80	8.42 ± 0.07	7.00	8.80
DekaPhan®	pH[a]	8.35 ± 0.10	6.00	9.00	8.50 ± 0.09	5.50	9.00
	specific gravity[a]	1.00 ± 0.00	1.000	1.015	1.00 ± 0.00	1.000	1.030
	leucocytes [leu/µl][b]	0	0	10-25	0	0	0
	nitrites [0, +, ++][b]	0	0	+	0	0	0
	protein [g/l][b]	0.30	0	5.00	0	0	5.00
	glucose [mmol/l][b]	0	0	0	0	0	55.00
	ketones [mmol/l][b]	0	0	1.50	0	0	1.50
	urobilinogen [µmol/l][b]	17.00	norm	17.00	norm	norm	17.00
	bilirubin [0, +, ++, +++][b]	+	0	+	0	0	+
	blood [ery/µl][b]	0	0	50.00	0	0	250.00
	haemoglobin [ery/µl][b]	0	0	0	0	0	250.00

[a] mean value±SE, [b] modus value, + light positive, Min minimum, Max maximum, n number of samples

Values for urinary pH (no matter which indicator papers were used), specific gravity and other urine parameters did not differ within alpaca farms ($p > 0.05$), except for proteins ($p = 0.04$). The urine of healthy alpacas should be negative for proteins (Fowler, 2010). However, proteins were detected in 35 urine samples of alpacas. Nevertheless, the results could be influenced by interference (Erba Group, 2015).

It is known that the pH of urine is influenced by the animal diet (Sundra *et al,* 2004), so the results are in accordance with the fact that the diets of alpacas were very similar within farms. On the other hand, in camels, both test strips showed differences in pH within zoos ($p = 0.04$ for both tests). However, multiple comparison tests showed no concrete differences between individual zoos in case of DekaPhan®. The results obtained *via* Duotest® suggested that the differences could be caused by different supplementary feeding in the form of grains or vegetable, not by green fodder. Also, the specific gravity as measured by DekaPhan® and the presence of ketones were influenced by the zoo in camels.

In both species, none of the measured parameters showed a significant difference between pregnant and non-pregnant females ($p > 0.05$), which supports the same conclusion derived from measurements of urinary pH of cattle by Veena and Narendranath (1993) and Dilrukshi and Perera (2009). But in the study of Skálová *et al* (2017), urinary pH was higher in pregnant heifers of cattle than in non-pregnant ones. Our results are incompatible with the hypothesis of Banerjee *et al* (1981) that pregnancy can be recognised by a change in the colour of camel urine.

In camels, the values of specific gravity obtained *via* hydrometer were higher than those obtained using the DekaPhan® strips ($p < 0.01$). However, DekaPhan® measurements can be influenced by pH values (Erba Group, 2015). The specific gravity obtained by hydrometer was always positively correlated with urinary pH, independent of whether Duotest® ($R = 0.66$, $p < 0.01$) or DekaPhan® ($R = 0.38$, $p = 0.01$) was used. Correlation between these parameters, even negative correlation, has been observed in humans (Shaafie *et al,* 2012). The specific gravity was also positively correlated with the protein content ($R = 0.52$, $p < 0.01$). The majority of the values obtained by hydrometer correspond with expected specific gravities for healthy camelids (Fowler, 2010). However, the results suggested that it is not possible to use urinary specific gravity for pregnancy diagnosis as was previously stated by Banerjee *et al* (1981).

In conclusion, it is possible to use accessible diagnostic test strips intended for simple human urinalysis for non-invasive indicative health control in camelids *via* selected parameters. Moreover, our findings do not support previously published results about usage of urinalysis for pregnancy diagnosis in camels.

Acknowledgements

We thank Iva Skálová for her cooperation and the Internal Grant Agency of the FTA CULS Prague (project no. 20185013) and CULS Prague University Internal Grant Agency (project no. 20175006) for funding this research and the article production.

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DOI : 10.5958/2277-8934.2018.00043.7 Vol 25 No 3, p 307-309

EFFECT OF DIFFERENT LEVELS OF AZOLLA (*Azolla pinnata*) INCORPORATION IN PELLETED COMPLETE FEED ON GROWTH PERFORMANCE OF CAMEL CALVES

Renu Kumari, R.K. Dhuria, N.V. Patil[1] and R.K. Sawal[1]

Department of Animal Nutrition, College of Veterinary and Animal Science, Bikaner, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India
[1]National Research Centre on Camel, Bikaner, Rajasthan, India

ABSTRACT

Fifteen healthy camel calves of nearly similar age (2-2 ½ year), body weight and uniform conformation were selected and distributed randomly into three treatments *viz.*, T_1 (control), T_2 (2% Azolla incorporation), T_3 (4% Azolla incorporation). All the pelleted complete feeds formulated for various treatment groups were prepared iso-nitrogenous and iso-caloric with concentrate to roughage ratio 60:40 with Azolla incorporation. Feeding trial was carried out for period of 60 days. There was no significant difference in DM Intake and digestibility of nutrients. Average body weight and body weight gain were similar in different treatment groups. The average daily live gain (ADG) was significantly ($P \leq 0.05$) higher in the treatment group than in control group. It could be concluded that non conventional feed resource i.e. Azolla can be utilised successfully in the feeding of camel calves by blending with other reliable ingredients in the form of pelleted complete feed. Feeding of Azolla to camel calves improve growth performance and feed conversion efficiency without affecting the feed intake and nutrient digestibility.

Key words: Azolla, digestibility, feed conversion efficiency, growth performance, pelleted complete feed

Scarcity of feed and fodder is one of the major impediments for sustainable livestock development and animal production in India; particularly for the resource-poor farmers in the rural areas. The feed deficiency has been the most important drawback in the production of livestock which accounts for nearly 50% of all losses in Indian animal production (Birthal and Jha, 2005). It is expected that the deficiency of 65% of green fodder and 25% of dry fodder will exasperate Indian livestock by 2025 (Singh *et al,* 2013). Conventional feed ingredients, particularly the protein supplements are expensive and are not readily available at affordable prices. The cost of conventional protein supplements is escalating with dwindling availability, and there appears to be an impending need to evolve an appropriate alternative for ruminant feeding (Kumar *et al,* 2012). Azolla is one of the aquatic plants with high biomass and protein production; it could be used as low cost feed source to meet the protein requirement of livestock. Azolla fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae* making it an excellent source of protein for livestock. This could be used as a part of pelleted complete feed for livestock. Supplementation of Azolla has been reported to improve the performance of lambs (Das *et al,* 2017), chicks (Yadav and Chhipa, 2016), bucks (Kumar *et al,* 2016), growing goats (Kumar *et al,* 2015) and buffalo calves (Indira *et al,* 2009). The present study was, therefore, conducted to evaluate the effect of Azolla containing pelleted complete feed on performance in camel calves.

Materials and Methods

Fifteen healthy camel calves of nearly similar age (2-2½ year), body weight and uniform conformation were selected and divided randomly in three treatment groups of five each *viz.*, T_1 (control), T_2 (2% Azolla incorporation), T_3 (4% Azolla incorporation). Feeding trial was conducted for period of 60 days to assess the effect of Azolla incorporation in pelleted complete feed on growth performance. All the pelleted complete feeds formulated for various treatments were iso-nitrogenous and iso-caloric with concentrate to roughage ratio of 60:40 in the mixture with Azolla incorporation. Body weight and body

SEND REPRINT REQUEST TO RENU KUMARI email: dr.renubijarniya@gmail.com

measurements of the experimental animals were recorded at fortnightly intervals to assess live weight changes and average daily gain.

Results and Discussion

The data on body weight, body weight gain and average daily gain of experimental camel calves are given in Table 1, 2 and 3, respectively. The mean fortnightly body weights of camel calves was found to increase from 367.4 to 392.96 Kg with overall mean of 380.10 Kg in T_1, 368.94 to 397.26 Kg with overall mean of 382.61 Kg in T_2 and 371.73 to 406.20 Kg with overall mean of 389.47 Kg in T_3 group, respectively in 60 days of experiment. The overall body weights at different fortnight intervals were found to be 369.36, 376.21, 384.14, 391.82 and 398.81 Kg at 0, I, II, III and IV fortnights, respectively. The mean fortnightly body weight gain were found to increase from 5.34 to 6.12 Kg with overall mean of 6.39 Kg in T_1, 6.14 to 7.52 Kg with overall mean of 7.08 Kg in T_2 and 9.07 to 7.36 Kg with overall mean of 8.62 Kg in T_3 group, respectively in 60 days of experiment. The overall body weights at different fortnight intervals were found to be 6.85, 7.93, 7.67 and 7.00 Kg at I, II, III and IV fortnights, respectively. The finding of present investigation is in accordance with the earlier reports of Reddy *et al* (2009) and Wadhwani *et al* (2010). The total body weight gain was 6.39 kg, 7.08 kg and 8.62 kg, respectively in T_1, T_2 and T_3 treatment groups with ADG of 426.00g, 472.00g and 574.44g.While, irrespective to treatment, overall average daily gain at different fortnight intervals were found to be 456.74, 528.44, 511.41 and 466.67g/d at I, II, III and IV fortnights, respectively. The overall mean values for DM intake (Kg/d) in different treatment groups i.e. T_1, T_2 and T_3 were observed to be 6.09, 6.41 and 6.66 Kg/d, respectively. While overall dry matter intake at different fortnight intervals *i.e.* I, II, III and IV fortnights, were found to be 5.75, 6.54, 6.71 and 6.54 Kg/d, respectively. The palatability score (Kg/100 Kg BW) were observed to be 1.60, 1.68 and 1.71 Kg in T_1, T_2 and T_3 groups, respectively. While, overall dry matter intake at different fortnight intervals was found to be 1.54, 1.72, 1.73 and 1.66 Kg/100 Kg BW at I, II, III and IV fortnights, respectively. The palatability score (g/ $KgW^{0.75}$) were observed to be 70.61, 74.13 and 75.95 in T_1, T_2 and T_3 groups, respectively. While, overall dry matter intake (g/$KgW^{0.75}$) at different fortnight intervals were found to be 67.76, 75.95, 76.79 and 73.76 at I, II, III and IV fortnight intervals, respectively. Body weight gain was higher in treatment groups compared to control group indicating more nutrient retention which might be due to supplementation of Azolla as it is a good source of nitrogen as well as different macro and micro-minerals (Kumar *et al,* 2015). Statistically average live body weight in T_1, T_2 and T_3 treatment groups were comparable but were similar to each other. Whereas, body weight gain and average daily weight gain were recorded highly significant difference between treatment groups. Indira *et al* (2009) also reported improvement in daily weight gain in buffalo calf with 50% replacement of groundnut cake nitrogen by Azolla supplementation. Similar results have been recorded earlier in lamb (Wadhwani *et al,*

Table 1. Effect of Azolla incorporation in the pelleted complete feed on body weight change in camel calves (Kg).

Fortnights	Treatment groups			Mean
	T_1	T_2	T_3	
0	367.40	368.94	371.73	369.36[a]
I	372.74	375.08	380.81	376.21a[b]
II	380.58	382.05	389.78	384.14ab[c]
III	386.84	389.74	398.84	391.82b[c]
IV	392.96	397.26	406.20	398.81[c]
Mean*	**380.10**	**382.61**	**389.47**	
SEM	**Treatment = 4.215**		**Period = 5.442**	

*Means with different superscripts in a column differ significantly.

Table 2. Effect of Azolla incorporation in complete pelleted feed on body weight gain of camel calves (Kg).

Fortnights	Treatment groups			Mean
	T_1	T_2	T_3	
I	5.34	6.14	9.07	6.85
II	7.84	6.97	8.97	7.93
III	6.26	7.69	9.06	7.67
IV	6.12	7.52	7.36	7.00
Mean*	**6.39a**	**7.08a**	**8.62b**	
SEM	**Treatment = 0.366659343**		**Fortnight = 0.423381741**	

*Means with different superscripts in a row differ significantly

Table 3. Effect of Azolla incorporation in pelleted complete feed on average daily gain of camel calves (g).

Fortnights	Treatment groups			Mean
	T_1	T_2	T_3	
I	356.00	409.33	604.89	456.74
II	522.67	464.44	598.22	528.44
III	417.33	512.89	604.00	511.41
IV	408.00	501.33	490.67	466.67
Mean*	**426.00[a]**	**472.00[a]**	**574.44[b]**	
SEM	**Treatment = 24.444**		**Period = 28.225**	

*Means with different superscripts in a row differ significantly

2010) and kids (Ghodake *et al,* 2012), when Azolla meal was supplemented in the basal diet at the rate of 20 and 15%, respectively. Chaterjee *et al* (2013) also recorded significant effect on average daily gain in cattle calves supplemented with Azolla meal. Similarly, Roy *et al* (2016) also observed improvement in growth performance in heifer fed on Azolla at 5% level in the concentrate mixture. Dry matter intake was observed statistically similar among the in treatment groups in the present investigation on supplementation of Azolla pinnata in complete feed. It is in accordance with the earlier reports (Kumar *et al,* 2012; Chatterjee *et al,* 2013) on effect dry matter intake on supplementation of *Azolla microphylla* in feed of male calves and does fed complete pellet diet containing 25% dried Azolla (Kumar *et al,* 2016). Similar observations have also been recorded in lactating cow (Sharma, 2013) and heifers (Roy *et al,* 2016) on supplementation of Azolla. Further dry matter intake was also observed to be similar in kids fed diet containing complete pellet feed containing Azolla (Kumar *et al,* 2017).

Conclusion

Looking to the observations recorded in present investigation, it could be concluded that non conventional feed resource i.e. Azolla can be utilised successfully for feeding of camel calves by blending with other ingredients in the form of a pelleted complete feed. Feeding of Azolla to camel calves can improve growth performance without affecting the feed intake and digestibility of nutrients. Further, inclusion of Azolla (*Azolla pinnata*) at 4 per cent level in pelleted complete feed could be viable proportion to keep pace with scarcity of feed and fodder to some extent and to have profitable camel rearing.

Acknowledgements

The authors are thankful to the Director, ICAR-NRCC, Bikaner for providing their infrastructure and scientific skills in carrying out the research work.

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SELECTED RESEARCH ON
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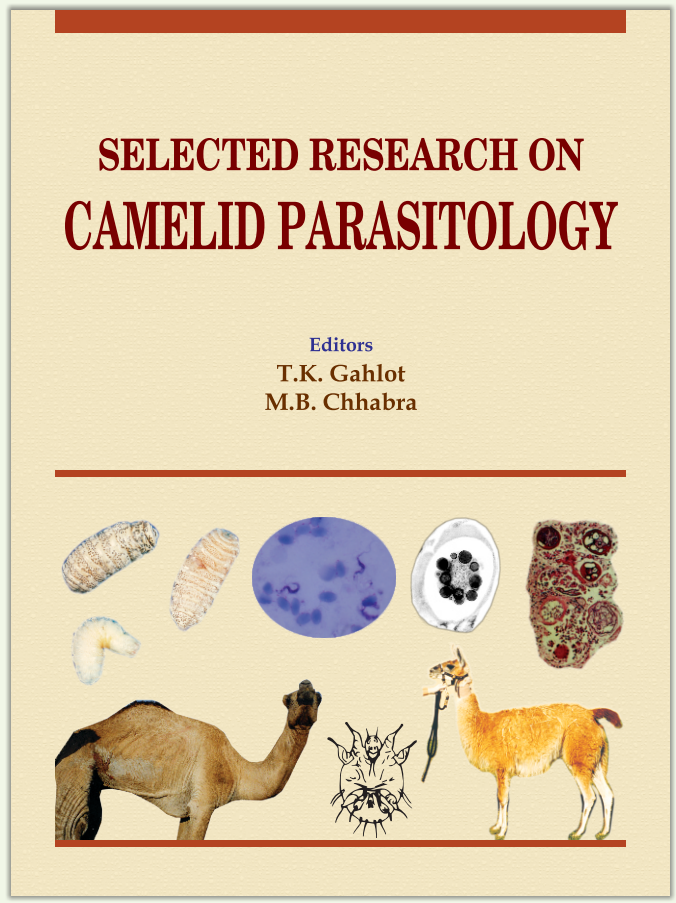
Hard bound, 291 pages, few figures coloured

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.



Editors:
T.K. Gahlot and M.B. Chhabra

Edition: 2009

Publisher: **Camel Publishing House**
67, Gandhi Nagar West,
Near Lalgarh Palace
Bikaner 334001 Rajasthan,
India
email: tkcamelvet@yahoo.com
website: www.camelsandcamelids.com

Price: US$ 200 (Abroad)
INR 3000 (India)

ISBN: 81-903140-0-9

DOI : 10.5958/2277-8934.2018.00044.9 Vol 25 No 3, p 311

Short Communication

ADHESIVE PLEURISY OF BOTH LUNGS IN A DROMEDARY CAMEL CAUSED BY *Streptococcus agalactiae*: A CASE REPORT

U Wernery, J Kinne, S Anas and J John
Central Veterinary Research Laboratory, P O Box 597, Dubai, United Arab Emirates

Streptococcus (Str.) agalactiae is an obligate bacteria of the mammary gland of cattle and camels and a well-known agent of chronic contagious mastitis. This microorganism may also cause occasionally neonatal septicaemias, kidney and uterine infections in dogs and cats (Markey *et al,* 2013).

A carcass of a 350 kg female, non-pregnant, non-lactating dromedary in poor condition was presented to CVRL for necropsy. The owner reported that he saw the camel in lateral recumbency before it died 2 hours later.

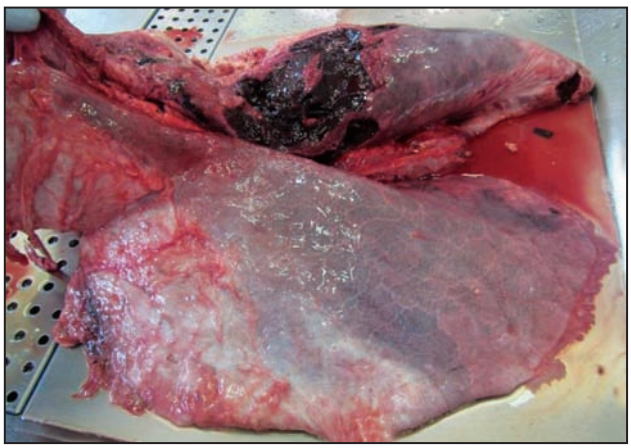


Fig 1. Adhesive pleurisy of a camel caused by *Str. agalactiae.*

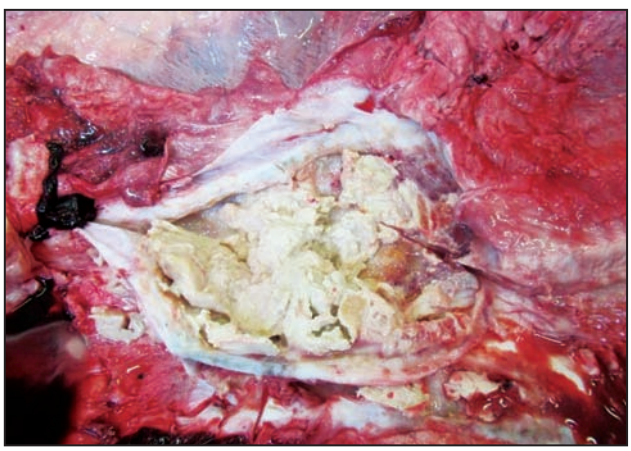


Fig 2. *Str. agalactiae* abscess containing necrotic brown mass with 3 cm indurated capsule.

During necropsy it was observed that both lungs were glued to the costal pleura and it was impossible to remove the lungs from the rib cage without loss of lung tissue (Fig 1). Additionally, on the right side of the lung attached to the ribs, a 20 cm long and 10 cm wide abscess containing necrotic brownish mass with severe capsule induration was found (Fig 2). From both lesions, the adhesive pleurisy and the abscess, *Str. agalactiae* was isolated in pure culture as well as from spleen and lung indicating a septicaemia.

Fig 3. A conglomerate of 5 kg of plastic (plastic lith) from C1.

There were no external lesions found in this dromedary camel. However, when the compartment 1 was opened, 5 kg of solid plastic was found (Fig 3).

In summary, it is believed that this dromedary camel contracted a *Str. agalactiae* sepsis including a severe adhesive pleurisy with abscess formation due to the ingestion of plastic.

References

Markey B, Leonard F, Archambault M, Cullinane A and Maguire D (2013). Clinical Veterinary Microbiology, Mosby Elsevier. pp 121-145.

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RESEARCHERS PRODUCE ALPACA ANTIBODIES USING YEAST

The new method, by contrast, produces the nanobodies using yeast cells *in vitro*. Using the DNA sequences of camelid nanobody genes, the team created a library of hundreds of millions of nanobodies, each variant attached to a different yeast cell. Fluorescent markers were used to attach target proteins to identify only the cells displaying nanobodies that would bind to a particular peptide.

Although not the only attempt to circumvent the need for a camel, the current approach is faster and more effective than previous methods, and is the first system made freely available for other research groups. A study coauthor Conor McMahon of Harvard Medical School says that making nanobody is quick and easy, we hope our platform will dramatically accelerate the potential applications of this exciting technology.

(Courtesy: The Scientist)

SAUDI UNIVERSITY DISCOVERS GENETIC CHARACTERISTICS OF ARABIAN CAMELS

A team from the Camel Research Center at King Faisal University, in cooperation with the University of Nottingham, has discovered the most important genes responsible for the mechanism for determining the colours of Arabian camels.

The study included analysis of 10 kinds of Arabian camels. According to the director of centre Dr. Faisal Al-Mathen the results were published in an international British scientific journal and reflected the history of the camels biological and genetic diversity in Arabia.

The study will reveal Arabian camels' mechanism of transmission of genetic traits, and will play a major role in the genetic classification of camels in the future.

(Courtesy: Arab News)

THE SAUDI CROWN PRINCE CAMEL FESTIVAL CELEBRATES THE REGION'S MOST POPULAR EXPORT

At the Crown Prince Camel Festival 2018, racing began on August 11, with the camel categories Mafarid, Haqqa, Laqaya, Jatha'a, Thanaya, Heil, Zamoul and Soudaniyat all classifying camels according to age, sex, and range of distances covered. The festival had three phases. The first phase ended on August 27 and the second phase continued until September 7. The festival then paused until September 15, when the final stages began and continued until September 27. The key races took place on weekends and included two sets of warm-up rounds, with 308 rounds of racing in part one and 178 in part two. In addition there were two marathon rounds, 20 production rounds and 178 closing-in rounds. Local and international camel owners competed for total prize money of SR45 million ($12 million) at the event. The festival also featured sports, cultural and entertainment activities alongside educational workshops for camel owners and visitors interested in camel sports.

For more than 30 days in August and September, business leaders, politicians and camel enthusiasts flock to one of the Middle East's largest displays of the finest camels in Saudi Arabia and the world. They compete in more than 700 racing rounds and beauty competitions, putting the festival firmly on top of the annual fixtures to celebrate Saudi culture, sport and the value of the camel. Early inhabitants of the Arabian desert relied on camels for milk, meat, leather, transport and also during battle due to their agility and speed: Camels can reach up to 40 miles an hour in short sprints. A camel can also run at 20 miles an hour for more than an hour without rest.

DOI : 10.5958/2277-8934.2018.00045.0 Vol 25 No 3, p 313-319

FINANCIAL VIABILITY OF RANGE LAND BASED CAMEL PRODUCTION SYSTEM IN ARID REGION OF GUJARAT

Khem Chand, BL Jangid[1], Subhash Kachhawaha[2], Ramesh Bhatti[3] and Pankaj[4]

Indian Grassland and Fodder Research Institute, Jhansi (U.P.), India

[1]ICAR-Central Arid Zone Research Institute, Regional Research Station, Pali-Marwar (Rajasthan) 306401, India

[2]ICAR-Central Arid Zone Research Institute, KVK, Jodhpur, (Rajasthan) 342003, India

[3,4]SAHAJEEVAN, Kachchh, Gujarat

ABSTRACT

The study of characterisation and economics of range land based camel production system uses primary data of randomly selected 75 camel breeders' households in Kachchh region of arid Gujarat. Range land based camel production system was the main occupation for more than 90 per cent of sample households with average herd size of 40 camels. Net returns per camel herd per year were found to be INR 62,687 with B: C ratio of 1.52. The Range land based camel production system was found financially viable enterprise with a payback period of 6 years, positive Net Present Value (NPV), and Internal Rate of Return (IRR) of 41.31 per cent. Camel production is declining in Kachchh region mainly due to degrading range lands, alternate employment opportunities in newly established industries, and market factors, hence needs strategies for sustainable camel production.

Key words: Breeding, camel, cost, investment, management, marketing, returns

Camel (*Camelus dromedarius*) production and its various uses is a major source of income for Raika/kachchhi muslims (*Maldharis*) community in Kachchh region of arid Gujarat. Camel can survive and reproduce under a low inputs management system, harsh environmental conditions and difficult landscapes in arid and semi-arid regions where survival of other animals is usually at risk (Schwartz, 1992; Kohler-Rollefson, 1997). It has a unique ability to convert the poor plant resources of the desert into milk, meat and fibre (Rathore, 2001). Hence, Camel breeding enterprise is still a one and only source of livelihood for many families of camel pastoralist communities in arid Gujarat. The use of camel for transportation of goods, building material, farm produce in different regions of Rajasthan is a common practice and thousands of families earn their livelihood from this enterprise (Kaushik *et al*, 1991; Kohler-Rollefson, 1992; Gahlot and Chada, 2000). However, the continuously decreasing population of camel in the state of Gujarat and the country (Govt. of India, 2014) needs strategic measures. Kachchh region of Gujarat had 22 per cent of Gujarat camel population during 2012. It would be important to understand the camel production system, resource use and its socio-economics in order to plan strategies for the communities who depend for their livelihood on this animal. Present study was aimed to analyse the production system and financial viability of camel breeding enterprise and policy interventions needed to improve livelihoods of camel breeders in Kachchh region of arid Gujarat.

Materials and Methods

A multistage stratified sampling technique was used to draw the sample (n-75) for this investigation in Kachchh district. Further 10 villages in this region having at least 15 camel breeder's population were selected in second stage. In the third stage all the camel breeders from these selected villages were listed and 75 camel breeders were selected using stratified random sampling technique. Beside camel breeders, 50 traders and other stakeholders were also selected for collecting information on marketing aspects. The secondary data were collected from various reports and state animal husbandry department of Gujarat state. Primary data from 75 sample households were collected during 2010-12. The information related to investment on camel and other permanent items, if any, used by breeders, feeding practices followed round the year, supplemental feed given, if any, health management

SEND REPRINT REQUEST TO KHEM CHAND email: kcmamnani@gmail.com

of animals, age of sale of camel calves, place of sale, value of animals sold, losses if any etc. was collected from selected respondents. Both personal interview technique and group discussion method was followed for collection of information. Key informants interviews were also conducted to know in details the camel breeding practices.

Beside data from breeders, information from 50 respondents involved in camel trade viz. traders, farmers, and carters were collected to know the age group of camels sold in the market, different uses for which camels are purchased, age wise price of camel and to identify the marketing channels used in its marketing. Standard enterprise budgeting methods were used for calculating variable and fixed cost of camel production (Johl and Kapoor, 2005). The financial viability of camel production was assessed using parameters such as Net present value (NPV), Pay-back period, Internal rate of return (IRR) and Benefit-cost ratio (BCR); computed using the standard methods (Gittinger, 1982).

Results and Discussion

Composition of sample camel herds

Camel keepers in the study area mainly rear female for reproduction purpose and sale young male calves with age of 1 to 2 years. On an average a camel breeder had 40 units of camel in arid Gujarat, where females were more than 50 per cent (Table 1). Camel herders sale young male calves produced in the herd at the age of 1 to 2 years.

Table 1. Composition and value of camel in sample herds (INR/ herd) n=75.

Particulars	No.	Value (INR)
• *Male*		
< 1 yr	3.48	19836
1-2 yr	2.25	18788
2-4 yr	1.05	15330
> 4 yr	0.40	8180
Sub total	**7.18 (17.78)**	**62134 (10.61)**
• *Female*		
< 1 yr	3.88	18815
1-2 yr	4.06	32485
2-4 yr	4.28	54332
> 4 yr	20.99 (51.97)	417738 (71.35)
Sub total	**33.21 (82.22)**	**523370 (89.39)**
Grand total	**40.39 (100.00)**	**5,85,503 (100.00)**

Note: Fig in parenthesis indicate percentage to Grand total.

Investment pattern

Fixed investment on a camel herd mainly comprised of animals, as its share in total investment was more than 99 per cent (table 2). The camel herders keep bare minimum items with them as they frequently move from one place to another. The proportionate investment on equipment & bedding etc was found to be 0.7 per cent only. It is evident from the investment that cost of female animals was the most important component of total fixed capital investment. Breeders of arid Gujarat did not invest on housing of animals because they stay in forest or common area or farmers' field for grazing. In case of equipments only few utensils were kept for collecting milk and making tea while BHAKAL (vernacular name of carpet made of camel wool) was also kept for using as bed during night.

Table 2. Investment pattern on camel herds (INR/ herd).

Items	Amount (INR)	Per cent
A. Animals	5,85,503	99.3
i) Male	62,134	10.5
ii) Female	5,23,370	88.7
B. Manger & Enclosure	0	0.0
C. Equipment, beddings & others	4,410	0.7
Total	**5,89,913**	**100.0**

Resource use pattern and management

Land utilisation pattern

The operational holding size of camel breeders in arid Gujarat was found to be 1.52 ha and land had no irrigation facility. Rajput and Tripati (2009) reported 13 per cent camel owners in landless category and according to Patel *et al* (2008) traditional camel breeders in Kutch district of Gujarat had very low land holding. Kohler-Rollefson (1992) pointed out that Raika gradually being forced out of their traditional occupation because of their landlessness. Camel breeders generally kept this land fallow as they were more dependent on income from sale of camel calves and camel milk and camel was not reared on stall feeding.

Housing management

Any housing for camel rearing is not practiced by the camel rearers. In all the selected areas of arid Gujarat round the year camel remains in forest/common areas/rangelands. In rainy season animals were kept inside the forest land, hillocks due to waterlogging problems in rangelands. To check the movement of camels in the night time

the front legs of animals generally tied with a piece of rope but breeding male camel was generally kept free. Regarding personal dwellings only 39 per cent families had Katcha houses made of mud and wood structures while other live in open lands by cleaning some areas surrounded by *Prosopis juliflora* shrubs or tress in forest or rangelands. During survey the living condition of camel breeders, specially, the *MALDHARIS* of Katchchha region was found very poor as small children and women had to live in open rangelands without any protection from extreme weather conditions. Rajput and Tripati (2009) reported that majority of Raika families (46.67%) were residing in mixed type of houses followed by those spending their live in kutcha or mud houses (27%).

Human labour utilisation

Human labour component is one of the most critical component in the camel rearing occupation. On an average proportion of family and hired labour recorded was 80 and 20 per cent, respectively. Mainly adult male labour (1.25 units) was employed for camel rearing, since, the animal herd was to be kept outside village round the year. The role of women members was significant in case of *MALDHARIS* (Camel breeders from muslim community) as they move with male members at new grazing sites and live with whole family in temporary shelters. While in other communities their role was almost negligible. Labour was hired, in case the herd size was big and family labour was not sufficient to care for it or it was mainly needed during calving season (mainly winter) as extra manpower was required to look after the young calves. Besides, maximum camel trade take place in winter due to organisation of livestock fairs in this season, help of hired labour was required for transporting animals to fair site or if the herd owner himself was moving to fair site for sale of animals or take care of animals, hired labour was kept at village to look after the remaining animals of the herd. Saini *et al* (2006) also reported similar practice in various camel rearing areas of Rajasthan. Rajput and Tripathi (2009) mentioned role of male and female members of Raika families in various outdoor as well as indoor camel husbandries related activities. The male members of Raika families performed work like taking camel to grazing pastures, training to camel, milking, treatment of sick animals through indigenous preparation, grooming, watering, cutting and transportation of fodder for camels etc. whereas the involvement of females in such activities was found almost negligible.

Breeding and calf management

Natural breeding was in vogue for camel production in this region and for a herd of 30-40 female one bull camel for breeding maintained. As average number of adult females in the herd was about 11 to 21 animals, herd owners shared the use of breeding camel bull to economise on cost account. Chand *et al* (2010) reported that in arid region of Rajasthan one breeding male was kept for 50 females in a camel herd. The breeding camel was replaced after 4 years to prevent inbreeding in the herd, thus they were preventing the disadvantage of inbreeding. The breeding bull camel was selected on the basis of well-built body, mother's milk yield (pedigree of mother), body colour, hump thickness, thin skin, long and thin leg, size of chest pad, scrotum position, length and development etc. Camel breeders of this region preferred animals of Kachchhi breed (Local breed in Kachchh region) as animals of this breed fetched better price in market. Camel calves were reared with herd and separate system of calves rearing was in practice.

Feeding/ browsing management

The camel breeders in Kachchh district of Gujarat reared two different breeds of camel i) Kachchhi camel and ii) Kharai camel. The camel herds were managed on extensive system of grazing/browsing and there were major three types of management system for camel rearing in Arid Gujarat. In first system camels were reared in Banni grasslands area near and around the Chari-Dhand wetland conservation reserve located on the edge of arid Banni grasslands. Chari-Dhand is a seasonal desert wetland and only gets swampy during a good monsoon. The camel herds in this system were mainly the kachchhi breed of camel; however, few herds of Kharai camel were also existed. In second system of management major source of forage was mangroves in the sea and trees/ bushes in the common lands. The camel herds of this system exclusively constituted by kharai breed of camel, it can swim in sea water for 2-3 kms. In third management system animals for fodder were depended on trees/ bushes in fallow land of farmers' field, forest area and common lands. The animals in these herds were mix of some animals of Kachchhi breed and other was of Kharai breed. The major sources of feed/fodder in all the systems were Salvadora persica Wall. (*Khara Jaal*), *Salvadora oleiodes* (Meethi jaal), *Maytenus semerginata* (Vekado), *Acasia nilotica* (Desi babool), *Ziziphus nummularia* (Jharberi) *and Ziziphus moritiana* (Ber), *Clerodendron*

phlomidis (Arni), *Launea spp.* (Chekudi), *Cressa cretica* (Oin), *Suaeda species* (Lano), *Acasia senegal* (Kumat), *Azadiachta indica* (Neem) and Mangroves etc. The major sources of water for animals was sweet water lake, rainwater collected in mangroves, village water ponds, tube wells, village cattle water troughs etc.

The intensive discussions with camel breeders of Kachchh region indicated rapid decline in grazing land available for camel. Camel breeders do not have access to traditional grazing lands, which are now under the jurisdiction of forest department. Village gaucher lands (Common grazing lands), were also declining due to encroachment as well as degradation due to lack of community management. Due to heavy industrialisation in Kachchh region particularly in Mundra, Lakhpat and Bhachau, mangroves, an important source of food for Kharai camels, are drastically reducing. Some rebari breeders give their camels to *jat* muslims (MALDHARIS) for grazing on monthly charge of Rs 50-80 per camel while they join other occupations.

General upkeep of animals

For drinking water animals were generally taken to village ponds and lakes etc. Camel were also taken to water structures (*AVALA*) made by farmers for drinking water to their animals. Frequency of watering to camel was thrice in a day during summer season while in winter season it was once or twice in a day. During rainy season animals were not taken to water source as they were free to drink water collected at various places in forest area/ rangelands. An adult camel required 20-40 litres of water per day. Animals were generally milked in the early morning and during evening time. The camel breeders staying around towns were able to sell the camel milk to tea stall and restaurants near highways. The milk was also procured by diabetes patients in the surrounding towns due to its medicinal value. Wool shearing activity was done collectively like a festivity and one person could shear wool of 5-6 camel in a day.

Migration pattern of camel breeders

In Kachchh region camel breeders migrate within tehsils as well as within the district during stress period (summer & winter) in search of forage and water for their animals. During severe drought years they migrate outside to the district/ regions towards Mehsana, Patan, and Saurastra region. In Bhuj tehsil, camel breeders from Pachchham move towards west Banni villages (Bhitara, Luna, Hajipir, Bhagadiya and Chachala) due to the greater availability of fodder and water, and come back to their native area in the rainy season. Some breeders from Lakhpat and Bachau shift to kiro hill and the surrounding villages of *Chhari Dhandh*, a natural wetland. Rebari breeders from Nakhatrana are known to migrate to the vagad region (Eastern Kachchh) of Rapar tehsil, whereas breeders from Mundra also migrate to nearby villages bordering the tehsil.

Health management

In arid Gujarat camel breeders did not take prophylactic measures for their camel and as a result sometime serious health problems occurred in Kachchhi and Kharai camels. The most prevalent diseases in this region were Trypanosomiasis, manze/skin infection, arthritis, gastrointestinal disorders, abortion and some respiratory infections. The occurrences of digestive problems viz. rumen impaction, abdominal pain and enteritis were found high in the herd. According to discussions held with camel breeders group and key informants, the use of veterinary services was estimated only less than 5%. A large proportion of owners provide health care to their animals using herbal rededicates or consulting traditional healers. The breeders sometime based on their experience directly procure drugs from private veterinary drug shops.

Marketing

The animals in this region were mainly sold through traders that were further sold in different livestock fairs conducted in different parts of Rajasthan and Gujarat. Few individual buyers like carters and sheep rearers also buy the camel directly from breeders. The practice of selling camel milk was not common in selected region. It was used by breeders for self consumption, for calves and only few camel breeders were bringing camel milk daily in nearby towns for sale to tea stalls or patients suffering from diabetes, tuberculosis, barol, cancer and asthma. There was no market for sale of camel wool in Arid Gujarat and breeders used this wool for making some items as veno, kurekhi, pariyar, anhoti (for preventing milking by calves), daman (small piece of rope for tying legs), bhakal (carpet) and different threads.

Cost and returns in camel production

Fixed cost

Average fixed cost worked out per year for a camel herd in arid Gujarat was INR 75,350. The share of interest in the total fixed cost was around two third (Table 3). As animals' cost was highest in the total

investment on herd, fixed cost of the animals was the major item of fixed cost (table 3). These results are in contrary to bovines where farmers spend on animal sheds and utensils and that also contributes to fixed cost.

Table 3. Fixed cost/ year in camel production (INR/ herd).

Particulars	Amount (INR)	Per cent
I. Interest (12%)	51639.33	68.53
• Animals	51110.13	67.83
• Others	529.20	0.70
II. Depreciation	23710.63	31.47
• Animals	22828.63	30.30
• Others	882.00	1.17
Total	**75349.97**	**100.00**

Note: Depreciation on animals is calculated for adult females with Junk value of INR 2, 500/- only. The normal age of female is 20 years and depreciation is taken from 5 year onward i.e. for 16 years. As young animals appreciate in value and male are generally sold, so no depreciation is taken for these animals. Depreciation on other items like utensils etc is taken @ 20 per cent per annum.

Maintenance cost

The average cost of maintaining a camel herd in arid Gujarat (40 animals) was found to be INR 1, 19,532. The proportion of fixed cost was more than 50 percent in total cost of maintaining a camel herd. The higher share of fixed cost was contrary to bovines where variable cost had higher share in the total cost as these animals were stall fed while in case of camel management labour is the major component with more than 25 per cent share in the total cost (table 4).

Returns

Average net return worked out per herd per year was Rs 62, 687 in Arid Gujarat (table 5). Family labor income per camel herd per year was Rs 87, 200 with B: C ratio of 1.52, it indicates that camel rearing was a profitable venture in this region. The sale of animals had maximum share in the returns. Camel breeders of this region sell male/ female calves of more than one year old to fetch better price in the market. Rajput and Tripati (2009) reported that the income of majority of the respondents (55 %) ranged between INR 3000 to INR 5000 per month; about 13% of the families had their earning even less than Rs. 3000, it indicated low income status of camel rearing households.

Financial viability of camel production

The financial viability of camel production was worked out using both undiscounted and discounted

Table 4. Maintenance cost per camel herd per year (INR).

Particulars	Amount (INR)	Per cent
1. Variable cost		
A. Grazing charges to forest department	2139.00	1.79
B. Material cost	5,967.81	4.99
Fodder	0.00	0.00
Concentrate, oil, and other miscellaneous expenditure	5967.81	4.99
C. Veterinary/health care Expenditure	5433.06	4.55
D. Labour cost	30,641.76	25.63
Labour for grazing & Gen Mgt.	30,000.00	25.10
Wool shearing	641.76	0.54
Total variable cost (A+B+C+D)	44,181.63	36.96
2. Fixed cost		
A. Interest	51639.33	43.20
B. Depreciation	23710.63	19.84
Total fixed cost (A+B)	**75349.97**	**63.04**
Total cost (1 + 2)	**1,19,531.59**	**100.00**
Family labour cost	24513.40	

measures. The Payback period worked out was six years for arid Gujarat. The camel production was financially viable at 12 per cent discount rate in terms of both NPV and BCR criteria, as NPV was positive and BCR greater than one. The IRR that indicates the maximum paying capacity of the camel rearing was estimated to 41 per cent for arid Gujarat. It implies that it would be financially viable to invest in camel production. The annuity value of camel production was INR 2, 83,894 in this region that indicates income generating capacity of the camel enterprise. Gross B:C ratio calculated was also found higher than unity confirming that camel breeding enterprise was profitable in this region. Thus it is clear that it would be financially viable and remunerative to the livestock rearers to invest in camel production. Thus based on different criteria it is obvious that traditional camel rearing enterprise despite several hardships to breeders was financially viable in both regions of Rajasthan. However the small ruminant rearing is quite attractive alternative as compared to camel rearing in western Rajasthan (Kumar and Upadhyay, 2009). Hence there is need for appropriate strategies to increase net returns from camel production for its sustainability.

Note: Indian National Rupees (INR) can also be expressed as Rs. or ₹

Table 5. Returns from camel (INR/ herd/ yr).

Particulars	Amount (INR)	Per cent
A. Sale of animals & value addition in calves	1,27,752	70.11
B. Other income	54,467	29.89
• Milk value	50,917	27.94
• Estimated value of wool	3,550	1.95
Gross returns	1,82,219	100.00
Total variable cost	44,182	
Fixed cost	75,350	
Total cost	**1,19,532**	
Net returns	**62,687**	
Returns over variable cost (ROVC)	1,38,037	
B:C ratio	1.52	
Family labour income	87, 200	

Table 6. Measures of investment worth per camel herd (INR).

Particulars	Value
1. Pay-back period (years)	6.00
2. Net present value at discount rate of 12 per cent (INR)	20,21,221
3. Internal rate of return (IRR) (%)	41.31
4. Annuity value at 12 per cent discount rate	2,83,894
5. Gross benefit-cost ratio at 12 per cent discount rate	2.80

Camel marketing

Marketing of camel was studied by collecting information from 75 breeders and 50 traders and carters in the study region. Majority of camel breeders sold male calves between 1 to 2 years as it was their main source of income. As there were few dairies based on camel milk in Gujarat, recently calved female camels were purchased by persons operating these dairies. The important marketing channel observed in the region was sale of animals through traders. These traders were buying more than 80- 90 per cent animals from camel breeders. These traders were further selling these animals to farmers, carters, tourist operators in different livestock fairs held in Rajasthan or Gujarat state. About 10-20 per cent animals were purchased directly from the breeders by camel dairies and carters.

The important marketing channels observed in both regions were as follows

- Breeder → Trader → Farmers/Carter/Sheep rearers (More than 90 % in Gujarat)
- Breeder → Sheep rearers
- Breeders → Carter/Farmer
- Breeder → Trader → Meat industry
- Breeders (Milk) → Hotels/Tea stalls

Constraints perceived by the camel breeders

Camel breeders' constraints were analysed using Garret ranking technique. The major constraints and their ranking has been shown in table 10. Lack of browsing area was the most important constraint mentioned by camel breeders in the regions. Prior to ban on entering in forest area camel breeders were free to browse their animals but after restriction they are not allowed and it was their major demand to open the forest lands for camel browsing as they were dependent on these lands for browsing since ages. The decline in camel population in the region was also due to the declining grazing areas; as these animals were not reared under stall fed conditions due to high feed cost and survive on forage available at trees/ bushes either in forest or range lands. Lack of health care services/ veterinary help for camel, also perceived as major constraint in the region. The major income source for camel breeders is sale of animals, but due to uncertainty in price of animals they do not have assured income. Lack of interest in young generation was also a constraint; as management of camel is very difficult and one has to follow the animals for 20-30 kms in a day for browsing. Breeders faced great problem in arranging hired labour for this occupation as young generation do not want to work with camel as they have to stay away from the family and other alternate employment opportunities are available at village itself. Opening of many industries has also diverted camel breeders of Kachchh region to work in these factories rather than living with camel for long period and stay away from their families. Wetlands, where camel feed on mangroves are under threat from heavy industrialisation along the coast (Arid Gujarat), further traditional grazing areas also degrading day by day and quality and quantity of available feed resources under stress. Further, as returns data indicated value of milk consumed or sold had a significant share in income, camel rearers faced problems in its sale in this region due to scattered population The absence of direct market for wool sale was also a constraint as breeders either have to throw the wool or prepare carpets (BHAKAL) with the help of rural artisans.

Conclusion and policy recommendations

The traditional camel breeding enterprise in arid Gujarat was found to be profitable and important source of gainful employment for camel breeding

households. But it was less attractive on account of drudgery involved. Comparatively lower returns from camel breeding might be the main reason for decline in population of the camel in the state and the country.

Table 7. Constraints perceived by camel breeders in arid Gujarat.

S. No.	Common constraints	Garrett's ranking
1.	Restrictions to grazing land under forest department jurisdiction	I
2.	Decreasing grazing land areas for camel	II
3.	Lack of health care services/ veterinary help for camel	III
4.	Uncertainty in market price of camel	IV
5.	Lack of interest in young generation (Rebari community)	V
6.	Wetlands, where camel feed on mangroves, are under threat from heavy industrialisation along the coast (Arid Gujarat)	VI
7.	No assured avenues for milk sale	VII

The declining interest of youth is mainly due to hardship and drudgery associated with the existing camel production system, changing social dynamics and scarcity of feed resources from commons are the other major drivers of this change. Blanket restriction on entry of camel in the forest areas is another major constraint for camel breeding, especially during rainy season (main crop season) as the options for grazing in this season are very limited. There is an urgent need for a strategy for enhancing sustainability of the camel breeding enterprise which is important to meet the critical demand for these special animals used by security forces, farmers and carters in the arid and semi-arid tracts of western India. The following interventions and measures are likely promote sustainable management of camel production system and improve camel breeders' livelihoods:

i) Participatory arrangements that allow camel grazing in allocated forest areas could help mitigate the fodder scarcity constraint. Such collaborative arrangements involving forest department, camel breeders groups and animal husbandry department might prove to be a win-win situation for all the stakeholders.

ii) The convergence under employment guarantee programme-MNREGS for sustainable management of the degraded common pasture like *ORANS* and *GOCHARS* would also help alleviate the feed scarcity problem for the camels.

iii) Enhanced opportunities to sell camel milk through mobile milk collection vans using cooperative network would increase camel breeders' revenue and provide regular income to meet daily family needs.

iv) Provision of mobile veterinary facility by state animal husbandry department in camel breeding areas would help in minimizing the losses due to various diseases.

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NASAL GENE SPRAY INSPIRED BY LLAMA ANTIBODIES COULD PREVENT ALL TYPES OF FLU

The spray, containing a virus engineered to make a protein derived from the llama antibodies, has passed its first animal test, protecting mice from every known flu strain that infects humans, a research team reports. The Janssen group injected llamas with a vaccine containing three different influenza viruses, as well as the viral surface protein, hemagglutinin, from two other flu strains, to create nanobodies against the flu. They then harvested four antibodies that each neutralised many flu strains. Ultimately, the team was able to engineer a gene that expressed a protein made up of nanobodies derived from all four antibodies. They spliced the gene into a benign adenovirus-associated virus (AAV) that's used in gene therapy experiments. Immunologist James Crowe, an influenza antibody specialist and vaccine developer at Vanderbilt University in Nashville, cautions that human immune systems may see the llama-derived proteins as foreign and develop antibodies against them. He also notes that although AAV-based treatments are being tested for life-threatening diseases, giving the virus as a flu preventive would face more intense scrutiny from regulators.

(Courtesy: News from Science)

AUSTRALIAN FERAL CAMEL POPULATION ESTIMATED TO BE AT 1.2 MILLION AND GROWING FAST

More than 1.2 million feral camels are raising havoc across Australia and they're spreading further afield every day. Baffled farmers confirm they have been spotted unusually far south — in the southeast coastal district of Western Australia. It is understood the nomadic desert beasts are migrating away from dry conditions in the Nullarbor and Goldfields in a desperate attempt to find food and water.

According to the latest Australia State of Environment Report (ASER), camels were introduced to Australia around 1840 and by 2008, an estimated 1 million camels were roaming the central arid lands of Western Australia, the Northern Territory, South Australia and Queensland. According to the Department of Biodiversity, Conservation and Attractions, the state is now home to 45 per cent of the nation's camels. According to the ASER, the "major impacts" of this burgeoning camel population includes damage to native vegetation and wetlands, increased competition with native animals for food, shelter and water resources, and damage to infrastructure and road hazards. Despite culling efforts between 2009 and 2013, which resulted in the deaths of 160,000 camels in Central Australia using ground-based and aerial culling techniques, the population has now swelled to around 1.2 million.

INDIAN SAFARIS HAVE BROUGHT BACTRIAN CAMELS BACK FROM THE BRINK OF EXTINCTION

Since the early 2000s though, the number of Bactrian camels in Nubra has increased—thanks to the residents of the Hunder village in the valley. In 2003, they decided to start camel safaris. As the initiative grew in popularity, the villagers formed the Central Asia Camel Safari, a registered cooperative society, in 2009. Other villages in the region, such as Sumoor, Diskit, and Tigger, also jumped on the bandwagon, forming their own camel unions. According to Current Science, there were around 150 camels in Nubra in 2012. Three years later, there were 211, including 21 calves below the age of one year. The camel population is largest in Hunder village, followed by Sumoor, Diskit, and Tigger. The residents of Hunder estimate there are currently around 250 camels in their village now.

(Courtesy: Quartz India)

DOI : 10.5958/2277-8934.2018.00046.2 *Vol 25 No 3, p 321-326*

COMPOSITION OF GHEE PREPARED FROM CAMEL, COW AND BUFFALO MILK

Nirav B. Parmar, Bhavbhuti M. Mehta and K.D. Aparnathi
Dairy Chemistry Department, SMC College of Dairy Science,
Anand Agricultural University, Anand, Gujarat, India

ABSTRACT

Samples of milk from camel, cow and buffalo were analysed for fat contents Cream was separated from the respective milk and ghee was prepared by employing direct cream method. Yield of ghee was measured and recovery of fat obtained was estimated. The samples of ghee were analysed for selected compositional attributes.Yield of ghee and recovery of fat from camel milk was substantially lower compared to that from cow milk as well as buffalo milk. The content of unsaponifiable matter, cholesterol and FFA of ghee from camel milk was significantly lower ($p>0.05$) compared to ghee prepared from cow as well as buffalo milk. On the other hand, moisture content of ghee from camel milk was intermediate to that of the ghee from buffalo and cow milk.

Key words: Buffalo, camel, camel milk, cholesterol, ghee

Ghee is one of the most important milk product manufactured and marketed in India and its subcontinent. Its origin dates back to prehistoric Indian civilisation as far as 1500 BC (HaeSoo *et al*, 2013). In some Middle Eastern countries similar kinds of products are usually made from goat, sheep or camel milk and are commonly known as samna in Egypt (Abou-Donia and El-Agamy, 1993), meshho in ancient Assyrian empire (2400 BC to 612 BC) (Abdalla, 1994), samin in Sudan (Hamid, 1993), maslee or samnin in Middle East, rogan in Iran (Urbach and Gordon, 1994) and samuli in Uganda (Serunjogi *et al*, 1998). Ghee is also gaining popularity in Australia, Arabian countries, the United States, the United Kingdom (UK), Belgium, New Zealand, Netherlands and many other African and Asian countries (Illingworth *et al*, 2009). According to Codex standard for milkfat products, ghee is a product exclusively obtained from milk, cream or butter, by means of processes which result in almost total removal of water and non-fat solids, with an especially developed flavour and physical structure (FAO and WHO, 2011).

Camel milk products are gaining popularity among the peoples and in future these products may be in a big demand (Mal and Pathak, 2010). It has been reported that camel milk is only suitable for drinking (Yagil *et al*, 1984). However, various products produced from dromedary camel milk include soft cheese, fermented milk, yoghurt, ice cream and butter (Al Haj and Al Kanhal, 2010).

The information on some compositional attributes, physical properties and chemical characteristics of ghee prepared from camel milk is very scarce. Hence, the present study was planned to compare ghee prepared from camel, cow and buffalo milk.

Materials and Methods

Collection of milk samples

The pooled milk samples (n=8, each species) of camel milk from Anand and Kheda district, cow milk and buffalo milk were collected from the local herd maintained in village nearby Anand. Samples were transported to the laboratory and stored at 4°C before its analysis. All milk samples used in preparation of ghee were analysed for fat contents of camel, cow and buffalo milk as per the method described in BIS Handbook (SP 18: part XI, 1981).

Determination of fat content in milk

The milk fat content in all the milk samples were estimated by following the Gerber method as described in BIS Handbook (SP 18: Part XI, 1981).

Preparation of ghee

Direct creamery method as reported by De (2004) was used for the preparation of ghee. Cream was separated from milk using cream separator. The cream so obtained was heated in a stainless steel vessel (pan) with continuous stirring till final

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temperature reached to 115°C for no hold. The ghee residues were allowed to settle and supernatant was filtered at 105°C through a four folded muslin cloth. The samples were weighed and filled in clean and dry glass bottles, cooled and stored to room temperature.

Estimation of fat recovery

It was estimated from amount of fat present in the milk taken for preparation of ghee and amount (yield) of ghee obtained at the end of the process.

% Recovery of fat = (Fat present in 100 ml milk/ Yield of ghee from 100 ml milk) x 100

Analysis of ghee for some compositional attributes

Moisture, unsaponifiable matter and free fatty acids (FFA) content of ghee were determined by methods as described in BIS Handbook (SP 18: Part XI, 1981).

The cholesterol content of ghee samples were determined by direct colourimetric method using Libermann-Burchard (LB) reagent. A calibration graph of absorbance was plotted against standard cholesterol and calculated the concentration of cholesterol in ghee.

Statistical Analysis

The data obtained during investigation were subjected to statistical analysis using completely randomised design (Rudolf *et al*, 2010). The data were averaged of 8 replicates for each type of milk.

Results and Discussion

Fat content of milk

The yield of ghee obtained from milk basically depends on fat content of the milk used for manufacture of ghee. The fat content of camel milk was between 3.8 and 5.81%, with an average of 5.07%. The average fat content of cow and buffalo milk were 4.30 % and 6.35%, respectively. The average fat content of camel milk was significantly lower than that of the buffalo milk, but significantly higher than that of the cow milk. Khaskheli *et al* (2005) reported that the amount of fat in camel milk ranged between 1.8 and 5.0 per 100 g, with an average of 2.63±0.40 g per 100 g. According to Al Haj and Al Kanhal (2010) fat content of dromedary camel milk was reported between 1.2 and 6.4% with an average 3.5±1.0%. In our earlier study, the average fat content in camel milk (Central Region of Gujarat), camel (Kutch Region of Gujarat), cow milk and buffalo milk was 4.35±0.11%, 2.92±0.16%, 4.65±0.08% and 6.57±0.20%, respectively (Yoganandi *et al*, 2015). Fat content of camel milk was reported to decrease from 4.3 to 1.1 per cent in milk produced by thirsty camels (Yagil and Etzion, 1980). The variation in fat content of milk from lactating mammals may be attributed to several factors including species of animal, breed of the animal, physiology of the animal, stage of lactation, number of lactations, season, feed, interval between the milking (Murtaza *et al*, 2017).

Fat recovery in preparation of ghee

Fat losses in manufacturing of ghee reduces the yield of ghee. It has direct impact on productivity of the operation and serious economic concern (Kumar *et al*, 2017). Therefore, yield of ghee and per cent recovery of fat were worked out on the basis of fat content of milk used for preparation of ghee and amount of ghee obtained from the milk.The yield of ghee obtained from the respective milk and corresponding recovery of fat are presented in table 1.

In preparation of ghee from camel milk average yield of 3.52 g/100g milk was obtained, giving the corresponding recovery of 69.43% fat from camel milk. On the other hand the recovery of fat was significantly higher in case of ghee from cow milk (88.84%) and buffalo milk (86.46%). The lowest yield of ghee was obtained from camel milk, in spite of having higher fat content compared to the cow milk. Purchase (1943) obtained 1.9 to 2.2g yield of ghee from 100 ml camel milk.

Table 1. Yield of ghee and recovery of fat obtained in preparation of ghee.

Sr. No.	Type of milk	Yield of ghee (g/100g milk)	Recovery of fat (%)
1.	Camel milk	3.52	69.43
2.	Cow milk	3.82	88.84
3.	Buffalo milk	5.49	86.46
	SEm	0.18	0.34
	CD	0.58	1.06
	Test	*	*
	CV %	9.88	0.94

It has been reported that in preparation of ghee by direct cream method from the cream containing 40 to 50% fat, recovery of 85% fat is obtained. (Anonymous, 2017). The low yield of ghee from camel milk compared to cow and buffalo milk might be attributed to greater losses of fat at various stages in the process. The poor recovery of fat in case of camel milk was primarily attributed to its poor skimming efficiency during separation of cream from the milk. Changade *et al* (2006a,b) studied different methods of

ghee making using cow and buffalo milk and their effect on the recovery of fat as well as found out fat losses at various stages. They obtained 85.9 and 85.5 per cent recovery of fat from milk fat of cow and buffalo, respectively. Mal and Pathak (2010) prepared ghee from camel milk through curd at 40°C and 45°C, churning curd into butter and clarifying the butter by heat. The yield of ghee was 2.25±0.14% and 2.57±0.23% for at 40°C and 45°C which was used for souring of milk. Therefore, yield of ghee from camel milk in the present study was well corroborated with results reported in the literature.

Khan and Appanna (1967) reported difficulties in extracting fat from camel milk using some traditional methods such as churning sour milk, likely because fat globules were firmly bound to the proteins in camel milk. Similar views were also expressed by Farah *et al* (1989). The authors found that in 5 camel milk samples with a fat content between 2.5 to 3.8%, the butter fat content of skim milk obtained after the 2nd centrifugation varied between 0.2 and 0.9%. Cow milk separation in the same centrifuge yielded skim milk with a butter fat content of 0.1 to 0.2%. Attia *et al* (2000) suggested that relatively high amount of emulsifying capacity and a small size of fat globules in camel milk causes difficulty in creaming of camel milk. In fact, under identical centrifugation conditions, the total separation of bovine cream was obtained after only one centrifugation, while the dromedary skim milk was obtained after the 3rd centrifugation. This relative stability of camel milk emulsion is in agreement with Stocks law as confirmed by both spontaneous and forced creaming. In both processes, the droplets of camel milk fat maintained a certain integrity during sedimentation. Their globule with membranes would therefore present a very marked hydrophilic character, despite their lipid nature, enabling them to have different type of bridges between the aqueous and fat phases of the milk. Therefore, poor recovery of fat from the camel milk in preparation of ghee and associated low yield of the ghee were very well in line with the views expressed in the literature.

Compositional attributes of ghee

The physical properties of ghee is dependent on the chemical composition of the fat. Therefore, moisture, unsaponifiable matter, cholesterol and FFA content of three types of ghee were determined and presented in table 2.

The moisture content of ghee from camel milk was significantly lower than that of the ghee from buffalo milk, however, it was significantly higher than the ghee from cow ghee. The content of unsaponifiable matter, cholesterol and FFAs of ghee from camel milk were significantly lower than that of the ghee from cow milk as well as buffalo milk.

Moisture

Moisture is always present because it cannot be completely removed merely by boiling. The average moisture content of ghee from camel milk (0.248%) was significantly ($p<0.05$) lower than that of the ghee from buffalo milk (0.235%), however, it was significantly ($p<0.05$) higher than the ghee from cow milk (0.280%). The acceptable moisture content level of ghee is 0.5% under FSSAI standards (FSSAI, 2017) and 0.3% under Agmark standards (AGMARK, 1988). In European Union, the butteroil defines "a product obtained from milk, cream or butter byprocesses which eliminate the water and the dry non-fat extract with a minimum content of milk fat of 99.3% of the total weight and a maximum water content of 0.5% of the total weight, including ghee". The specifications for moisture content of ghee/similar products in other countries like US and Japan also in the same range (Lee *et al,* 2018). Therefore, the results of this study showed that in all 3 types of ghee moisture content was within the standards specified for ghee.

Cholesterol

The study on milk fat sterol fraction is of great interest from a nutritional view point. The study may also contribute to quality control of milk products, especially, fat rich dairy products (Goudjil *et al,* 2003). Cholesterol is the major sterol component of most milks with at least 95% of the total sterols (El-Agamy, 2006). In the present study, cholesterol content of ghee obtained from camel milk (0.168%) was significantly lower than that of the ghee from cow milk (0.286%) as well as buffalo milk (0.245%).

Sterols represent about 0.25-0.45% in total fat. Its contents depend among other things on the method of fat extraction and is believed to be near 0.4% in most cases. The main component of the sterol fraction is cholesterol [300 mg/100g of fat (Goudjil *et al,* 2003)].

It appears from the literature survey that very limited reports are available about cholesterol content of camel milk lipid. El-Agamy (2006) stated that cholesterol is the major sterol in camel milk fat. Gorban and Izzeldin (2001) determined the percentage of various lipid components of camel milk by laser densitometric scanner. The authors reported cholesterol content of 0.1%in esterified formand 0.84%

Table 2. Some compositional attributes of ghee.

Sr. No.	Attribute	Type of ghee			SEm	CD(0.05)	Test	CV (%)
		Camel	Cow	Buffalo				
1.	Moisture (%)	0.248	0.280	0.235	0.003	0.009	*	2.71
2.	Unsaponifiable matter (%)	0.375	0.528	0.450	0.006	0.019	*	2.41
3.	Cholesterol (%)	0.168	0.286	0.245	0.007	0.020	*	7.11
4.	FFA (% oleic acid)	0.125	0.136	0.153	0.008	0.025	*	13.45

in free form. According to Fahmy (2015) cholesterol in camel milk is lower than cow or goat milk.

Kumar *et al* (2010) reported a cholesterol content of 0.319% and 0.265% in cow ghee and buffalo ghee, respectively. Sankhla *et al* (2010) found cholesterol content in camel milk fat ranging from 0.097 to 0.182% with an average of 0.133%.

Unsaponifiable matter

Some of the constituents present in oils and fats are collectively known as unsaponifiable matter and usually composed of sterols, higher alcohols, tocopherols, hydrocarbon (mainly squalene), pigments and fat soluble vitamins. Some of these constituents may exert a pronounced effect on stability and nutritional properties of the oils and fats. Sometimes, characteristics of unsaponifiable matter help to detect adulteration of the oils and fats (Dhara *et al,* 2010). The average unsaponifiable matter content of ghee from camel milk (0.375%) was significantly lower than that of the ghee from cow milk (0.528%) as well as buffalo milk (0.450%). Since, cholesterol is the major component of unsaponifiable matter of milk fat, the content of unsaponifiable matter content followed the same trend as that of the cholesterol content of ghee of all three types.

Sankhla *et al* (2010) stated that the unsaponifiable matter content in camel milk fat ranged from 0.356 to 0.5% with an average of 0.404%. Bindal and Jain (1973) reported average unsaponifiable matter content of 463 mg% in cow ghee during winter and 428 mg% during summer. Similarly, in case of buffalo ghee the unsaponifiable matter content was 399 mg% during winter and 392 mg % during summer. Sankhla and Yadava (198l) reported the unsaponifiable matter content of 0.416% ± 0.02 in cowghee and 0.392% ± 0.07 in buffalo ghee. However, Sharma (1989) found unsaponifiable matter in cow ghee ranging from 0.458 to 0.562%; averaging 0.505%) and buffalo ghee ranging from 0.426 to 0.526%: averaging 0.472%. Therefore, unsaponifiable matter content of camel, cow and buffalo ghee was in agreement with the reports in the literature.

FFAs

A small portion of fatty acids not esterified in triglycerides is freely dispersed chiefly in the milk fat phase and slightly in the milk-water phase and is termed free fatty acids (FFAs). FFAs are usually present in low concentration in milk fat, however, they play very important role in flavour of milk and milk products. FFAs are formed mainly as a result of lipolytic enzyme action on glycerides. The FFAs content of ghee from camel milk were significantly lower than that of the ghee from cow milk as well as buffalo milk.

The buffalo milk fat contains less (0.22%) free fatty acids than cow milk fat (0.33%) (Khedkar *et al,* 2016). It appears from the literature survey that no data are available on the cholesterol content of camel ghee. Cardak *et al* (2003) determined out FFA content of camel, goat and cow milk using GC/MS. Milk samples were extracted by diethyl ether/n-heptane mixture (1:1 v/v) after addition of ethanol and 2.5M H_2SO_4. The FFA isolation was carried out with an aminopropyl column. The total FFA, concentration of camel milk was 13.64 µmole/10 ml milk and goat and cow milk a FFA content contained 13.53 and 22.56 µmol/10 ml milk FFA, respectively. Sankhla *et al* (2010) found the FFA content in camel milk fat from 0.24 to 0.39% with an average of 0.32%. Thus, FFA content of camel milk ghee was in accordance with the reports in the literature for camel milk fat.

Conclusion

The study undertaken on preparation of ghee from camel milk revealed that the yield of ghee and recovery of fat from camel milk significantly differed from the ghee prepared from milk of cow and buffalo. Similarly, moisture, unsaponifiable matter, cholesterol and FFA content of ghee from camel milk also differed significantly from the ghee prepared from cow and buffalo milk.

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AUTHOR INDEX

SUBJECT INDEX

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