Proceedings of the 3rd Conference of the International Society of Camelid Research and Development

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Foreword

On behalf of the Organizing and Scientific Committees, I am glad to present the Proceedings of the Third Conference of the International Society of Camelid Research and Development (3rd ISOCARD), which is being held in Muscat, Sultanate of Oman from the 29th of January to the 1st of February 2012. The Conference has attracted 200 participants from 39 countries. Over one hundred oral presentations will be given, and participants will have the opportunity to see approximately 85 poster presentations. We are sure that the selected topics will provide a wealth of information and many opportunities for discussions. The invited speakers' papers will be published in a special issue of the Journal of Camelid Science.

The theme for the 3rd ISOCARD Conference is "Challenges Facing Camelids in a Changing World". It includes various sessions, namely: physiology, biochemistry and pharmacology, medicine, health and infectious diseases, immunology, reproduction, anatomy and surgery, pastoral systems, genetics and biotechnology, meat and products, milk and nutrition. The latest research findings by scientists from a broad range of camel research bodies around the world, including research, government agencies and industry are included in the proceedings. They reflect important advances pertaining to camelid health and production. We trust the proceedings will promote international communication in camelid science.

I would like to thank all the authors who have contributed to the publication

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Genetics and Biotechnology

Medicine, Infectious Disease and Health

Reproduction

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Pastoral Systems

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Genetics
and
Biotechnology

1. Molecular Characterization of Kachchhi Camel *(Camelus dromedarius)* Using Microsatellite Markers

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Introduction

Camel genetic resources of India consist mainly of single humped (*Camelus dromedarius*) and a few double humped camels (*Camelus bactrianus*). There are eight recognized camel breeds in India viz., Bikaneri, Jaisalmeri, Marwari, Mewari, Jalori, Mewati, Kachchhi and Malvi (www.nbagr.res.in/regcamel.html). The Kachchhi breed is a good milk yielder and is probably the only Indian breed adapted to marshy land. Distribution of Kachchhi camels is restricted to Gujarat state; their habitat encompasses Kachchh and Banaskantha districts. The 2007 Livestock Census of the Government of India shows drastic decline in the camel population, in the last decade were 29,920 Kachchhi camels. However, some recent survey by a local agency reports only 13,483 Kachchhi camels in the Kachchh district (Das *et al.*, 2011). The rapid decline in the Kachchhi camel population warrants conservation. Characterization of breeds is the first step in the conservation programme. The microsatellite markers are considered as the most powerful genetic markers for characterization of plant and animal genetic resources (Goldstein and Pollock, 1997). The present study planned to investigate the genetic variation in the Kachchhi breed of camel using sixteen microsatellite markers.

Materials and Methods

A total of 74 blood samples were randomly collected from non-related animals belonging different areas of Kachchh district of Gujarat state aseptically into vacutainers coated with EDTA (0.5 mM, pH 8.0). A total of 16 microsatellite markers - VOPL03, YWLL40, LCA66, LCA63, YWLL44, VOPL08, VOPL32, YWLL59, YWLL38, VOLP67, LCA59, LCA56, YWLL29, YWLL08, YWLL36 and VOPL10 were used to assess the genetic variation in the Kachchhi breed. Genomic DNA was isolated from blood samples using standard phenol: chloroform extraction method (John *et al.*, 1990). These 16 microsatellite marker loci were amplified in five multiplex PCR panels. The amplified products were sized by fragment analysis on ABI automated DNA sequencer using GSLiz500 as size standard.

Results and Discussion

Out of the 16 microsatellite markers, 14 loci were found to be polymorphic whereas two loci YWLL40 & YWLL08 were monomorphic with a size of 172bp and 155 bp respectively. The number of alleles in the polymorphic markers ranged from 2 (VOPL32, YWLL59, LCA56 and YWLL29) to 7 (VOPL10). A total of 51 alleles were observed at 16 microsatellite loci. The observed and expected mean number of alleles (MNA) were 3.18 and 2.06 respectively. Comparable estimates are observed in other dromedary camel breeds e.g. 2-5 alleles in Jaisalmeri Indian camel (Gautam *et al.*, 2004), 2-7 alleles in Bikaneri Indian camel (Mehta *et al.*, 2007), 4-6 alleles in Baladi, Somali, Sudani, Maghrabi and Mowallad camels (Karima *et al.*, 2011).

As a measure of deviation from HW equilibrium, the Chi-square and likelihood ratio test showed a total of six loci with P-value indicating deviation from HW expectations. The F_{IS} values for these marker loci were positive except for LCA59 (-0.0163). The mean F_{IS} value of 0.1354 indicates sizable level of inbreeding in this breed. Although the range is wide, the mean observed and expected heterozygosity were 0.364 and 0.421 respectively. The values of PIC are lower than heterozygosity for the corresponding marker. The PIC values ranged from 0.206 to 0.711 with PIC more than 0.50 at only four loci. The low MNA and narrow allele size range observed in the present investigation could be due to use of less polymorphic markers and probably does not indicate lower genetic variability of this breed. Higher genetic variability was observed for this and other Indian camel breeds when other sets of microsatellites were used (Vijh *et al.*, 2007; Mehta S. C. personal communication).

Since the population of Kachchhi camel breed has been reduced drastically, genetic effects of reduction in population size require evaluation. The BOTTLENECK program was used to test for

genetic bottleneck in the recent breeding history of this breed (Cornuet and Luikart, 1996). Under the assumption of the stepwise mutation model (SMM), the most suitable model for microsatellite evolution, neither the sign and standardized differences tests nor the Wilcoxon Signed Rank Test revealed any significant result ($p > 0.05$). These findings indicated the absence of genetic bottleneck in the investigated population, and the population can be considered in mutation drift equilibrium. However, thetypical L-like distribution of the allele frequencies was not observed.

The present study contributes to the knowledge on population structure and assessment of existing genetic diversity in the Kachchhi camel population. Further genetic analysis of other Indian camel and their comparisons need to be carried out to determine the phylogenic evolutionary relationships and genetic distances among the indigenous camel breeds.

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2. Animal Genetic Resource Management by Camel Breeders in Ansongo Region, Mali

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Introduction

Several more or less recent evolutions threaten the pastoral livelihoods and the genetic diversity that they fostered and that supported them for centuries. In particular, the status of camel genetic resources is poorly understood, as well as the ongoing transformation of the livelihood systems that cradled them. In Mali, most the studies about camel breeding date back to the 1980's. Since 2000, the negotiated peace between government and the Tuareg rebellion gave rise to a renewed national interest in camel breeding. This survey aimed at identifying the camel herders strategies and constraints before further productivity assessment and genetic improvement studies. Aspects of mobility and genetic resource management are emphasized in the present paper.

Material and Methods

A survey was conducted from November 2010 to January 2011 among 100 camel herders in the Ansongo region, Mali, and covered 4 districts, differing by their distance to the Niger river and their ecological conditions. With an area of 23'614 km^2, 132'205 inhabitants and an arid to semi-arid climate, the Ansongo region officially harbors 28'380 camel heads. The districts of Talataye and Tin Hama are respectively located 180 and 60 km from the river (north-east); they are part of the so-called Hausa zone. The two other districts, Ouatagouna and Tessit, are part of the so-called Gurma zone; these are closer to the river and more humid areas. The questions addressed the major characteristics of the household, herd structure, breeding aims, constraints, practices and their rationale. A particular attention was paid to their strategy regarding mobility and genetic resources management through several open questions.

Results and Discussion

Livestock was the main activity for 95% of the surveyed households; half of them had no side activity. Herd management practice and production performance were markedly affected by their general remoteness. Across the whole region, over 90% of breeders had no regular access to veterinary services. Ethno-veterinary practice was well-developed and shared although some breeders lacked the necessary knowledge (5%). Beyond salt supplementation, which was widely used (95% of herds), nutritional supplementation with cotton seed oilcake was practiced by 18% of breeders. Located in Tin Hama and Talataye, the herders had benefited of their previous inclusion in an experimental protocol and adopted supplementation on that occasion. Vaccination and deworming were also more applied by these breeders for the same reason. As Talataye is most remote area, with movements continuous, irregular and involved the whole family (nomadism). It was also a district in which the use of veterinary services and nutritional supplementation was best adopted comes in contradiction with literature (Chaibou and Faye, 2005). The explanation provided here above suggests that awareness can partially circumvent the effects of remoteness or nomadism. The integration of camel breeding and agriculture through agreements for crop residues grazing was seldom practiced (13% of herds).

Besides the obvious rationale for mobility that are food and water seeking, other motivations were mentioned as participating to traditional festivals (25%) and avoiding conflicts with peasants (15%). Trade and social cohesion were also cited. A last common rationale for mobility was that it is a physiological need of the camel. Movements occurred all year round and annual distances were highly variable (10 to 500 km). The decisions regarding mobility (timing, route) were taken by the head of household in 58% of cases and were otherwise collective, involving both men and women. Movement concerned the whole household in Talataye while herd splitting was most commonly practiced in other districts. Labor was hired for camel herding in 27% of households. Insecurity along herding routes was cited as a major constraint to mobility.

Regarding genetic resources, the evolution of livestock portfolios was strongly divergent between districts, with a substitution of cattle to camels in the less arid zone of Tessit, while camel

were still the first choice of breeders in Tin Hama or Talataye. The description of camel types was different between the Hausa and Gurma zones. In the former, breeders described two main types: the Tilabayaten type, which is high and slender with good milking ability, and the Talmorokit, told to be smaller and best fit for transport. In the Gurma zone, numerous types were cited, most of the names then referring to the color rather than to the complete phenotype of the camel (Emalli, white; Akawal, black; Abzaw, dark grey; Ezagague, red; Awrague, yellowish; Azaref, brown). The multicolored Azarghaf was also present in the Gurma. Some particular cases were the Awinague, which refers to a white animal with vision problems, and the Adignas, meaning trust and referring to resilience. This classification gives a wider insight in camel diversity in the region compared to the four denominations reported by Ouloguem *et al*. (2004). The names Talmorokit and Tilabayaten are common to the latter and the present studies. The Azarghaf is a well-known phenotype present in the Sahel region, while the here-mentioned Abzaw seems close to the Abzin, studied by Chaibou and Faye (2004) in Niger.

Breeding management mostly consisted in the choice of breeding males. Only 15% of the breeders used males from outside, either by buying them or through agreements with other breeders. Citation frequency of selection criteria were similar (khi-square test; $p > 0.5$) between Hausa and Gurma zones. The first criteria cited were beauty (27.5%) and milk (25%), followed by work ability (19%) resilience (16%) with racing performance listed last (12.5%).

Conclusion

Mobility was substantially affected by factors such as insecurity, agricultural encroachment and climatic evolutions. Differences in mobility strategies were accompanied with differences in decision making in the household as well as different evolutions of livestock portfolios. Important evolutions of pastoral systems harboring camel genetic diversity are thus at play while almost no knowledge of this diversity is available. Part of this knowledge deficit is due to the difficulty of gathering correct information about this diversity and its management through household surveys. Interesting elements were collected in the present survey, as the distinct classification given by breeders in the Hausa and the Gurma zones. Focus groups might be used as a better tool to disclose the informational framework needed to establish the foundations of performance evaluation, diversity monitoring and genetic improvement.

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3. Anafi, Bishari and the Cross Breed: Sudan Racing Camels, A Review

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Introduction

The last estimation of camels population in the Sudan was about 3,908,000 heads (Ministry of Animal Resources, 2005). Camels in the Sudan are classified as pack (heavy) and riding (light) types according to the function they perform and probably as a result of selection applied for these traits by the various camel-owning tribes (Gillespie, 1962). The present study is literature review on phenotypic descriptions, owner tribes and area of Sudan racing camels.

Racing Camel Areas

Riding camels are restricted to the north-east of the country between the Nile and Red Sea. The Anafi type is generally found in Gadaref state (eastern Sudan); Gezira and Sinnar states (Central Sudan), while the Bishari camel is mainly found in the Eastern Sudan (Kassala and Gadaref states).

Owner Tribes

The Anafi breed is usually found in small numbers and raised with other types of camels. It is owned by Rshaida and Lahween tribes (Gadaref state), Shukria, Bataheen and Ahameda tribes (Gazira State), Rufaa and Kenana tribes (Sinnar state), Gaaleen, Hawaweer, and Hussania (in River Nile state). The Bishari camel is mainly found in eastern Sudan (Kassala and Gadaref states) and bred by Bishareen, Amarar, Beni Amir and Hadendowa tribes; it is also breed with other tribes (Shukria and Lahween) but in small numbers with other types (Sakr and Majid, 1990).

Phenotypic Descriptions

Anafi breed

Is a fast racing camel used for short distance races. It has a long head and erect ears, (Sakr and Majid, 1990). The white color is predominant in this breed, but animals with yellowish color are also found. The hair is short and soft and the hump is small, erect and located in the middle to the back. The females have small size udders and teats. Is also said to be a good riding animal, although not of outstanding quality since it is bred for speed rather than for stamina. It is less robust than the Bishari, but fast and smooth, having no rival for distances of up to 40km (Ishag *et al*., 2011).

Bishari breed

Is a famous for long distance racing. It has short, wide concave for head, Roman nose; short and strong legs (Sakr and Majid, 1990). This breed is distinguished by its white or yellowish coat color, short hair and concave face profile. The hump size is small to medium, located in the middle of the back with erect orientation. The udder and teats of Bishari camel are also characteristically of small size (Ishag *et al*., 2011)

Bashandi or (As-hab)

Is a Cross breed, known as good racing camel. This group is believed to be a cross between Alrabi type female with Anafi or Bishari male. It has a fine skeleton with fine legs, a medium body size with a mean live weight of adult males is 350 kg. It is distinguished by its short hair and white color (Sakr and Majid, 1990).

Table 1. Body measurements and weights of camel racing breeds in the Sudan.

Breed	Barrel girth (m)	Heart girth (m)	Height at shoulder (m)	Body weight (kg)
Anafi	1.32±0.04	2.86±0.02	2.75±0.01	315.84±12.20
Bishari	1.34±0.04	2.86±0.02	2.75±0.01	316.26±12.46

Source: Ishag *et al*., (2011)

Table 2. Camel speed of the two breeds (*Annafi* and *Bishari*), (Darosa and Agab, 2007)

Breed	3 km	6 km	8 km	10 km
Bishari	4.55 min.	9.55 min.	14.0 min.	18.30
Anafi	4.55 min.	10.30 min	13.50 min.	17.30

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4. Suggestions for Genetic Improvement of Camels in Sudan

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Introduction

We will attempt to offer some suggestions for genetic improvement of Sudanese camels. Camels of Sudan were estimated to be 4.406 millions (Ministry of Animal Resources, 2008); and Sudan is rated the second in camel population in the world. However, till now there is no genetic improvement policy adopted by camel owners in the Sudan. Improvement goals of camels must be matched with production objectives of the owners, the management potentials and with the prevailing environment. Therefore, production systems, production constraints and available infrastructure must be seriously considered in the planning and implementation of sustainable improvement program. Genetic improvement must be built on scientific facts and indigenous knowledge of camel owners.

Selection Objectives and Goals

Camel owners usually keep camels due to their appreciated productive potential and adaptability. Productive traits such as growth rate, milk yield and fertility have a high priority as they influence the sale of animals and the use of milk to satisfy family needs (Ishag and Ahmed, 2011). In addition, adaptive traits such as disease resistance, drought tolerance in addition to the low cost of breeding are extremely important considering the highly unfavorable production conditions in arid and semi-arid areas (Ishag and Ahmed, 2011). Consequently, it is important to keep the adaptive characteristics at their present level. Generally, genetic improvement goals include the improvement of meat and milk production (dual purpose animals with high growth rate and sustainable lactation milk yield), productive herd-life, tolerance to prevailing disease and fertility traits (age at first calving and calving interval). The traits related to growth are relatively easy to improve through a breeding program (mass selection) and usually have moderate to high heritability estimates (Hermas, 2009 and Alnajjar *et al.*, 2009). On the other hand, traits related to adaptation are difficult to measure and to select for. In any case, it is difficult to improve upon the present adaptability of most Sudanese camel breeds and hence the main aim will be to prevent any deterioration of adaptability traits. Production traits should be selected in the given production environment (Franklin, 1986), thus allowing adaptation to respond as a correlated set of traits as an option for improving both the production and the adaptation of animals (Horst, 1983).

Young sire breeding system

A young sire breeding system could be adopted in camel production systems and is already practiced. Breeding camels are selected from within young males based on information about performance of dam, sire and about their own production performance and evaluation. Enhancing these endogenous practices by introducing performance and pedigree recording and using available information about relatives, a young breeding camel program seems to be most appropriate. The tribal set up may be used as an advantage in to organize such a system. Since each ecotype is mainly raised by a specific tribe, the tribal authority and tribal elders can help in the selection and rotation of sires. A progeny testing scheme is organizationally not applicable, as it is too costly and time consuming.

Nucleus breeding schemes

Breeding programs will only be successfully implemented where accurate recording is possible. Accurate record keeping under field conditions requires financial means, expertise and well-developed infrastructure such as transport and communication structures. Due to lack of a recording system and a relatively small herd size, breeding programs must be built on alternative means of recording and on different selection methods. Open nucleus breeding schemes with controlled mating and the formation of pedigrees are widely suggested to circumvent the high costs arising from field performance recording and selection. The genetic progress in the nucleus as a result of recording,

selection and planned mating, can be disseminated to the participating herds through use of males originating from the nucleus. In such schemes, the best males are kept for breeding in the nucleus, while the remaining selected males are used for breeding in the commercial herds.

It will be necessary to establish four nucleus herds distributed over camels breeding regions: two in Kordofan, Darfour (western Sudan), one in Butana plain (eastern Sudan) and one in Sinnar state (central Sudan). These nucleus herds will contain mainly Arabi camels which are dual purpose animals (meat and milk). The number of females in each herd should be about 400 plus 10 males with 1:40 male: female ratio. The nucleus herd should be formed by selection of superior females and males from camel populations in the region on the basis of their performance and performance of dams and sires. The nucleus herd could be kept permanently in station or it may be allowed seasonal movement according to the station circumstances (availability of feeds and disease prevalence). Traditional natural mating would be practiced in nucleus herds and participants' herds. Breeding camels could be kept for a maximum of 4 years in herd to prevent inbreeding. The suggested open nucleus scheme may consist of three levels, the first level is the nucleus herd, and the second level is the propagation herds, while the third level is the herds of camels' owners that were not included in the propagation herds. The camel owners in the second level (propagation herds) are to be selected according to their herd size, willingness to participate and level of education. Also as far as possible they should be able to keep records. Breeding camels and young females not needed in the nucleus herds should be moved to propagation herds, while only superior females are to be moved from propagation herds to the nucleus herds. Breeding males and young females not needed in propagation herds should be transferred to herds in the third tier. Table 1 shows assumptions of reproduction and management parameters for genetic improvement of camels.

Table1. Reproduction and management parameters for genetic improvement of camels in Sudan

Parameters	Values
Sex ratio	1:1
Calving interval	2 years
Fertility rate	80%
Survival rate among calves	80%
Preselection for growth and appearance traits for males and females	60% and 80%
Pedigree selection rate for males and females	4% and 80%
Productive life of males	4 years
Replacement rate of males and female camels	30% and 20%

The nucleus herd with the above assumptions would produce a proximately 128 male calves and 128 female calves every year. On the basis of preselection 76 male calves and 103 female calves will remain in the nucleus. The best 3 and 80 young male and female calves have to be selected from among preselected calves to be used as breeding replacement in the nucleus herd. Since the size of the nucleus herd must remain constant, male and female camels are annually screened for comparative performance and other functional defects and replacements decided accordingly. The remaining 72 males and 22 females would be sold to the participants in propagation herds (2nd level). The size of propagation herds is assumed to be 4000 females and 100 males. Again preselection and selection will be practiced within this tier. Twenty Eight young males will be selected and added to those males coming from the nucleus herds (72) to be used as breeding males. The remaining males and females from this tier would be sold to participants or owners in the 3rd level.

The young sire breeding program have quick turn-over rate of male breeding camels compared with the progeny testing breeding scheme. On the other hand, half sib breeding program is affective as young sire program, while the high accuracy gain of this program compared to young sire program is nullified by prolongation of the generation interval. The young sire breeding program seems to be most adequate for camel breeding.

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5. The Role of Embryo Transfer in Accelerating Genetic Improvement in Lactating Dromedary Camels (*Camelus dromedarius*)

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Introduction

Dromedaries have not been specifically selected for high milk production. For this reason, average daily production is similar across different geographical regions, different breeds and management systems. However, there is significant individual variation in milk production among dromedaries (Juhasz *et al*., 2009). There are several reasons behind the slow genetic improvement for milk production. First of all, there are no reliable, long-term production reports of a large camel population that could be the base for genetic selection. Secondly, dromedaries are known to have low reproductive efficiency and pregnancy rates (40 %; Tibary and Anouassi, 1997). Thirdly, the calving interval is as long as 2.5 to 3 years due to ―lactation anoestrus‖, management practice (mating after weaning) and to the fact that camels dry off within 3 months of conception (Nagy and Juhasz, 2010). During the last 20 years, embryo transfer has been studied and applied in racing dromedaries (Skidmore *et al*, 2002).

The aims of this presentation are (1) to demonstrate the effect of early breeding on milk production, (2) to describe the benefits of embryo transfer in a dairy operation (3) and to show the results of our embryo transfer program using high producing and low producing dromedaries as donors and recipients, respectively.

Materials and Methods

Eleven, multiparous camels in mid lactation were selected for the 1st study. The camels were milked by milking machine twice a day and milk quantity was recorded. Ovarian activity was monitored with ultrasonography (Aloka 500, 5 MHz, Japan) at regular intervals. All camels were mated when the size of the dominant follicle reached 1.2-1.5 cm. Pregnancy was diagnosed by ultrasonography and progesterone determination. Average milk production of 2 week periods was compared from 2 months before until 2 months after conception. At the end of the 2 month period, 4 pregnant dromedaries were given PG F2-alpha (Cloprostenol, 500 μg/animal, i.m.; Estrumate, Schering-Plough, USA) to induce embryonic mortality. Production data of the entire lactation were collected and compared between pregnant and non-pregnant camels. The effect of pregnancy on milk production was tested with analysis of variance.

In the 2nd study, 10 high producing lactating dromedaries were selected as donors at the end of the breeding season. Follicular activity was monitored by regular ultrasonography. Donors were given 20 μg Buserelin i.v. (Receptal, Intervet, Holland). Starting on day 4 after GnRH, each donor was treated with a combination of 2000 IU eCG im**.,** (Folligon, Intervet, Holland) administered as a single injection and a total dose of 700 IU/400 mg, pFSH (Folltropin, Bioniche Animal Health, Ireland) twice daily in declining doses over a period of 4 days. Donors were mated with a fertile bull twice 24 hours apartwhen follicles reached 10 to 15 mm in diameter and embryo recovery was carried out on Day 7 after ovulation. Recovered blastocysts were transferred non-surgically into recipients that had been induced to ovulate 1 day after the donors. Pregnancy was diagnosed by ultrasonography and serum progesterone determination at 14, 21, 35, 60 days and 5 months.

Results and Discussion

In the 1st study, all camels had follicular development and were mated (20 cycles). Seven of 11 animals conceived 284 ±21.5 days post-partum. There was a significant effect of time (P<0.001), pregnancy (P<0.05) and interaction (P<0.001) on average milk yield. In non-pregnant dromedaries, milk decreased slowly over time. In pregnant camels, a slow decrease until Day 30 was followed by a sudden drop from 8.8 ±0.24 to 6.3 ±0.16 kg/day by Day 60 of gestation. Total milk production and length of lactation was significantly higher in non pregnant compared to pregnant camels (P<0.001).
In the 2nd study, average total milk production per lactation and daily yield were 3345 ±199.7 kg and 8.1 ±0.4 kg per donor (mean ±SEM), respectively. Superovulation was successful in 9/10 camels resulting in the development of an average of 19.6 ±2.8 follicles and 14.3 ±2.0 corpora lutea per

donor. A total of 56 embryos were recovered (6.2 ±1.5 embryos/donor) with significant variation in recovery rate between camels (12 to 76 %). Embryos were transferred into 46 recipients (36 single and 10 twin transfers) and pregnancy rate at 60 days was 34.8 % (16/46). Pregnancy loss between 21 to 60 days was 20 % (4/20).

We conclude that pregnancy significantly decreases milk production in dromedary camels. For this reason, early mating of high producing camels – in order to decrease calving interval – results in important milk loss. On the other hand, the long lactation period and late mating impede the genetic potential of these dromedaries. Embryo transfer is an excellent solution to overcome this problem. Multiple offsprings could be obtained from camels of high genetic potential during lactation without any adverse effect on milk production. Hence, we conclude that embryo transfer has a great potential and vital role in accelerating genetic improvement in lactating dromedaries.

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6. Status of Cloning by Somatic Cell Nuclear Transfer (SCNT) in Camels

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Cloning by SCNT has a special significance in the genetic improvement of camels and can be used to produce elite males; racing champions; animals with the highest potential for milk production, or the prized beauty camels. Optimization of the techniques for dromedary oocyte maturation (Wani and Nowshari, 2005, Wani and Wernery, 2010), ultrasound guided transvaginal ovum pick-up (Wani and Skidmore, 2010), chemical activation of mature oocytes (Wani, 2008), and in vitro embryo culture (Wani 2008, 2009) during the past few years was the basis for our recent success in production of world's first cloned camel, named Injaz, (Wani *et al.*, 2010). Injaz, who was born on April 8th 2009, has been produced from the embryo reconstructed with cumulus cell obtained from a slaughtered animal. However, our second cloned camel named Bin-Soughan, who was born on Feb 23rd 2010, has been produced from the embryo reconstructed with the skin fibroblast of an elite live bull. Live cloned offspring's have resulted from SCNT with cumulus cells, granulosa cells, oviductal, uterine, and ovarian epithelial cells, mammary gland cells, skin fibroblasts and blood cells in other animal species. The easiest and non-invasive method of harvesting the nuclear donor cells from an elite animal, however, remains to be either from its skin or blood. We have demonstrated that both cumulus and skin fibroblast cells from camel can be reprogrammed in reconstructed embryos and such embryos can not only develop in vitro but also lead to gestation and the birth of a cloned calves following embryo transfer. This has opened doors for the amelioration and preservation of genetically valuable animals by harvesting the donor cells from a small skin sample from such animals. We have also demonstrated, for the first time, that adult fibroblasts (from both cumulus cells or skin cells) can be cultured, expanded, and frozen without losing their ability to support the development of cloned embryos, a technology that may potentially be used to modify fibroblast genome by homologous recombination so as to generate genetically altered cloned animals. This technology can also be used to store the cells from valuable animals for possible use in SCNT or related techniques in future.

The potential applications of somatic cell nuclear transfer in camels are currently, however, constrained by low pregnancy rates from the transferred reconstructed embryos. Currently, the efficiency for nuclear transfer in animals including camels is between 0–10%, i.e., 0–10 live births after transfer of 100 cloned embryos. We have obtained better pregnancy rates from some cell lines, however, overall it is still low when compared to other assisted reproductive techniques. Many factors including recipient cytoplast source, their preparation, nuclear donor cell and their treatment, influence the success of cloning process. Presently, we have very little information about the fundamental molecular and cellular events that could be involved in reprogramming the nucleus of an adult somatic cell after embryo reconstruction and its activation. However, tissue of origin, age of donor, cell culture conditions and length have been shown to influence the development of reconstructed embryos. The objectives of our research concentrated on the optimization of the nuclear transfer procedure to make efficient use of the limited number of oocytes available in this species. We compared the use of in vitro matured oocytes obtained from slaughterhouse ovaries and in vivo matured oocytes obtained from stimulated donors by ultrasound guided transvaginal ovum pick-up for their developmental potential after embryo reconstruction by SCNT. We also compared different cell types and cell treatments in the development of reconstructed embryos in vitro, their in vivo development after transfer into recipient surrogate mothers and live births. Studies were also conducted on the synchronization of recipients and their management after cloned embryos were transferred to them. This presentation will discuss the present status of cloning by somatic cell nuclear transfer, the current challenges and the future strategies to be applied in order to enhance the use of this technology for application in camelids.

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7. Result and Shortcoming of Camel DNA Paternity Testing

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Summary

The dromedary camel (*Camelus dromedarius*) has played a key role in the history of man and civilizations. Beyond providing transport, meat and milk, the camel also serves as a means of entertainment and competition. An accurate method for paternity assessment has become of importance in the case of highly priced animals in the racing and breeding industry. This has put a lot of emphasis on establishing a registry for camel breeding.

Microsatellites are the markers of choice for a variety of genetic analyses including population genetic, linkage analysis, genome mapping as well as parentage verification and individual identification. A total of 110 published microsatellite markers from New World camelids (NWC) and Old World camelids (OWC), eight of which have been reported for dromedary, were assessed for their suitability for parentage verification in the dromedary camel. Efficient amplification was observed for 50 markers, a subset of which was used to create a panel of markers that are highly informative and undoubtedly reliable for parentage testing in camels. Implementation of this microsatellite panel in racing industry will be discussed.

Introduction

The dromedary camel (*Camelus dromedarius*) is one of the most economically important domesticated species in Arabian Peninsula, North Africa and Middle East. Camels have played a vital role influencing every aspect of daily life (Mariasegaram *et al.*, 2002; Spencer *et al.*, 2010). Beyond providing transportation and food, the camel also serves now a day as a mean of entertainment and competition. This has put a lot of emphasis on establishing an accurate method for paternity verification especially for racing and breeding industry.

Microsatellite markers are abundant and highly polymorphic sequences that dispersed throughout eukaryotic genome. Microsatellites are transmitted from one generation to the next through simple and stable inheritance. Accordingly, microsatellites are the markers of choice for parentage verification (Tozaki *et al.*, 2001).

Many microsatellite markers have been isolated from NWCs and OWCs; however, only eight have been reported, by CVRL, for the dromedary. A total of 110 published microsatellite markers from NECs and OWCs, Bactrian camel (*Camelus bactrianus*), llama (*Lama glama*), guanaco (*Lama guanicoe*) and alpaca (*Vicugna pacos*) as well as dromedary (*Camelus dromedarius*), were assessed for their suitability for parentage verification in the dromedary camel.

Material and Methods

A total of 200 camel blood samples were collected from UAE camel farms for evaluation of 110 microsatellite markers in this study.The data were analyzed and the polymorphic information content of efficiently amplified markers was calculated. The successfully amplified markers were assigned to different cocktails. The information generated from analysis of these markers was then used to create two panels for parentage verification.

Results and Discussion

Efficient amplification was observed for 50 markers of dromedary camel, three of which were monomorphic. Allele sizes, PIC, observed (H_o) and estimated (H_E) heterozygosity as well as probability of exclusion (PE) for candidate parent from parentage were calculated for each marker. The information generated a set of 34 microsatellite markers, two panels, which are highly informative and undoubtedly reliable for camel parentage testing.

The results reveal that these markers have effectively provided robust data that can be used to verify parentage. Since the camel genome has not been explored to a great extend, as in the case of equine and bovine, the new panels will ensure high integrity and pedigree information for the racing and breeding industry. So far, around 4600 samples, 100 hair and 4500 blood samples, collected from UAE, Saudi Arabia, Qatar, Kuwait and Oman camel farms, have been genotyped using these markers. This has contributed in formation of a database for camel parentage verification. This database is an invaluable tool for setting up a camel registry in the region which would assist breeders to maintain accurate pedigree records and minimize inbreeding in their herds.

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8. From the Bush to the Genome: Genetic Identification of the Last Wild Old World Camel Species *Camelus Ferus*

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Introduction

Species are groups of interbreeding natural populations that are reproductively isolated from other such groups (Mayr, 1995). The Biological Species Concept (BSC) is one of the most widely accepted definitions of a species. In the case of Old World camelids (*Camelini*), however, this concept cannot be applied appropriately. Despite their divergence five to eight millions years ago (mya), *C. bactrianus* and *C. dromedarius* still interbreed and generate fertile descendants over several generations. A third species in the ―Old World‖ is concerned of this specific feature: the Wild camels (*C. ferus*) are highly threatened by hybridization with their domestic relatives.

The last wild representatives of Old World camelids are critically endangered (IUCN 2010) and exist today only in small numbers (approx. 1600) in the cold deserts of Mongolia and China. For a long time they have been discussed very controversially to be either feral or truly wild and the ancestors of the domestic Bactrian camels. However, the International Commission of Nomenclature (ICN 2003) fixed the first available specific name based on a wild population ―*Camelus ferus*‖ for the Wild camel discovered by Przewalski in 1878, therewith classifying it as separate species (Gentry *et al*., 2004). Using mitochondrial and nuclear DNA analysis we give evidence for the genetic differentiation of Wild camels and identify them as separate species *Camelus ferus*.

Material and Methods

For the genetic differentiation between wild (n=94) and domestic (n=166) Bactrian camels we sequenced 804 bp of mitochondrial DNA (mtDNA) and analysed 19 microsatellite loci as described previously (Silbermayr *et al*., 2010a and 2010b). For the whole-genome analysis of a single Bactrian camel (Zoo Herberstein, Austria) we used 5µg DNA for paired-end read sequencing (2x101 bp) on a Illumina Genome Analyzer IIx. After trimming and quality check of the reads we created a *de novo* assembly using CLC Genomic Workbench. In the next step, we mapped the reads with BWA against the *de novo* assembled Bactrian camel genome and estimated basic population parameters like the population mutation rate $\theta = 4N_e\mu$ and the sequencing error rate using mlRho (Haubold *et al*., 2010).

Results and Discussion

The analysis of mtDNA revealed a monophyletic clustering of all Wild camels and a high genetic differentiation with the domestic Bactrian camels of 1.8%. This is comparable with previous analysis of wild and domestic Bactrian camel mitochondrial genomes (Ji *et al*., 2009) and with thedivergence seen in wild and domestic New World camelids (Silbermayr *et al*., 2010a). Similar high levels of genetic differentiation between wild and domestic Bactrian camels could be observed on the nuclear DNA level ($F_{ST} = 0.34$). It is important to note that we found 13 domestic/ hybrid camels among the 94 Wild camel samples collected in the strictly protected areas of Mongolia and China. The separation between the Wild and domestic Bactrian camel was estimated at 0.2 - 0.7 mya in the Pleistocene (Ji *et al*., 2009), long before domestication took place (4,000 – 5,000 ya). Consequently, we can exclude that the wild camel populations in Mongolia and China are the direct ancestors of their modern domestic relatives.

Contrary to other livestock species for which the genome is already known or currently studied (e.g. dromedary; Al-Swailem *et al*., 2010) nuclear genomic data from the domestic Bactrian camel have been missing so far. Using genomic DNA of a single Bactrian camel we created a *de novo* assembly obtaining 2 Gb genomic sequence corresponding to almost two thirds of the Bactrian camel genome With an average 5.3-fold sequence coverage we discovered 304,232 polymorphic single nucleotide polymorphisms and obtained a likelihood estimation of the population mutation rate θ of 1.29×10^{-3} with a sequencing error rate of 6.64×10^{-4}. Compared to other domesticated ungulates the observed nucleotide diversity in camels is higher than in cattle, but similar to pig.

Maximum Likelihood tree based on the mt-genomes of Camelini & Lamini.

We conclude that the Wild camels are a separate species based on monophyletic clustering and high genetic differentiation with their domestic relatives and we exclude them as direct ancestors of today's domestic Bactrian camel populations. We note that hybridization between these two species can be observed. Our results provide a basis for the *in-situ* conservation of Wild camels and for the investigation of selection under domestication and genome-wide association studies.
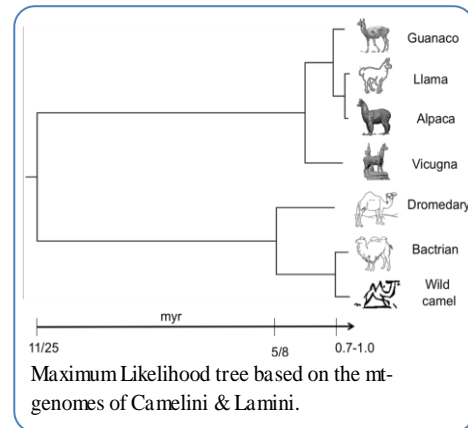
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9. Body Measurements of Saudi Arabia Camel Breed (*Camelus dromedarius*)

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Introduction

The total population of dromedary in the Arabian Peninsula was estimated at approximately 1.6 million camels, about 53% in Saudi Arabia (Al-Eknah, 2008).The Kingdom of Saudi Arabia is probably one of the main area where the dromedary camel was domesticated 5000 to 6000 years ago (Uerpman and Uerpman, 2002), and is the place where the camel biodiversity is one of the most important in the world. The selection for milk or meat or race purpose as well as the selection for coat color lead to a high variety of breeds and types which have been described by several authors. The present study aimed to classify the camel breed of Saudi Arabia on the base of their body measurements in an attempt to identify groups with similar conformation.

Material and Methods

Total of 152 camel owners were visited in 9 regions of the kingdom (Al-jouf, Arar, Tabuk, Tabarjal, Riyadh, Qassim, Hail, Jazan and Al-bahah). They were selected on the basis of variability in breed composition of their camel farm. In each farm, a questionnaire was applied and measurements were taken from female and male camels regarded by their owner as the more characteristic for a given breed. Data from 212 camels (155 female and 57 males) belonging to 12 different camel breeds or types were collected.

The measurements were taken on standing animals with a measuring tape in cm. The following measurement were taken: (i) the length of the head from nose to occipital (LH), (ii)The length of the neck (lower part) from base of head to the chest (LN), (iii) The circumference of the neck at the middle of the neck (CN), (iv)The height at the withers (HW), (v) girth circumference at the middle of the thigh (TC), (vi) The length of the left front teat (LT), (vii) The length of the udder from the front to hind attach (LU).

The mean of the different measurements was computed (Table 1). In a second step, a table including the 12 identified breeds (in row) and the different mean values of body measurements (in column) was analyzed by automatic clustering, achieved for assessing the proximities between the different breeds according to their mean body measurements.

Results

The Body measurements for female Saudi camels is given in Table 1 and their clustering is given in Figure 1.

Table 1. Mean body measurements of 12 types or breeds of female camel of Saudi Arabia (in cm)

Breed	Lhead	Lneck	cNeck	Lteat	Ludder	Height	GirthC	ThighC
Adhana	42.1	87.8	74.8	4.2	17.0	173.0	180.5	73.3
Aouadi	42.4	97.6	79.3	4.7	15.7	174.3	191.3	83.6
saheli	42.8	96.2	86.4	5.1	16.7	176.0	195.9	84.3
shageh	39.3	92.3	92.0	5.2	17.0	182.7	180.7	90.3
Awrc	41.5	92.0	88.8	4.6	18.5	199.3	201.0	88.8
Zargah	40.5	87.0	91.0	4.5	22.0	185.0	222.0	86.5
Asail	42.3	94.3	86.3	2.0	6.3	185.8	199.3	78.3
Homor	46.5	107.1	83.9	4.7	25.6	186.7	217.3	93.1
Majaheem	46.9	110.7	89.4	6.8	25.0	192.2	219.2	94.9
Shaele	46.9	104.5	83.0	4.1	24.8	187.0	213.5	86.9
Sofor	48.1	98.7	81.0	4.3	22.7	185.3	220.9	85.3
Wadda	47.4	108.6	79.0	4.8	25.4	186.7	221.8	93.0

Figure 1. Classification of the 12 female camel breeds of Saudi Arabia according to their body measurements showing four types of camels
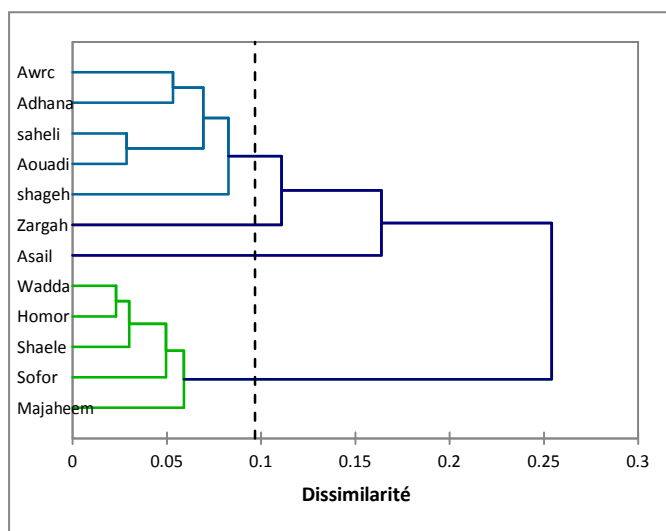
Discussion

The body measurements for phenotyping had been used in the camel (Ishag *et al*., 2011). Except for the thigh circumference, the body measurements are poorly correlated, i.e, relatively independent of the different parameters chosen. The classification of Al-Eknah is based on ecosystem (desert, hill, coast) or use (riding, racing or production), and the present phenotyping was close to this classification.

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10. A Comparative Study on Camel Breeds For Growth and Digestibility

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Introduction

The population of camels in the Arab world is nearly 11 million heads. Representing approximately 76% of the global total (19 million(. About 14% of the total animal units in the Arab countries. Camels are in most Arab countries, but they are concentrated in Somalia (56%), Sudan (24%), Mauritania (7%) and Saudi Arabia (3.5%).

Saudi Arabia has many camel breeds, spreading all over many regions. Camels in Saudi Arabia are classified according to their colors, and use for milk and meat production. The main types of camels in Saudi Arabia are Majaheem camel (black, dark color and high-lactation milk), Wodoh camel (a medium-sized, moderate in milk production), Suffr camel (large to medium-size, color mixture between the white and red) andSho'l camel (the colors overlap between red and blond).

There is no information on Saudi camel breeds regarding their meath production, nor their growth requirements or nutrition and husbandry.

This study was conducted to investigate the effect of Saudi camel breeds (Majaheem, Suffr, Sho'l, Wodoh) on growth and digestibility coefficients.

Materials and Methods

Young male camels of the Majaheem, Suffr, Sho'l, Wodoh breeds, 6-9 months old with average weight 133.83±2.83 kg. Animals entailed three animals in four replicates for each breed. A balanced energy/protein ration (Alfalfa hay and concentrate mixture 16% CP) were used to ensure that animals get their nutrient requirements using ad lib twice feeding system. The experiment lasted for 204 days. Feed intake for each group was recorded weekly and daily feed intake was calculated. The animal's weights were recorded every two weeks, before the morning meal. The daily gain weight and feed conversion ratio was calculated. At the end of trials, one animal from each replicate (four animals per breed) was used in the digestibility study.

Feed and feces were analyzed according to AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest *et al*. (1991).The data were analyzed according to SAS (1998).

Results and Discussion

The results obtained from the growth trial indicated that final weight of animals after 204 days was 291.78 ±2.53, 278.17 ±5.68, 283.56 ±10.95 and 269.61 ±5.35 kg for Majaheem, Wodoh, Suffr and Sho'l, respectively with an average weight of 280.78 kg. The average daily gain was 0.767, 0.698, 0.730 and 0.686 kg for Majaheem, Wodoh, Suffr and Sho'l respectively. Feed intake was 4.73 ±0.05, 4.68 ±0.05 and 5.24 ±0.42 and 4.53 ±0.13 kg for Majaheem, Wodoh, Suffr and Sho'l, respectively. Majaheem breed had the best feed conversion efficiency compared to other breeds. The camel is distinguished from other animals as it only needs small amounts of food to cover their requirements. It can also compensate for the loss quickly upon re-feeding back to the normal level (Wilson, 1984). The present results are in agreement with the results of Basmaeil (1989) and Farid *et al*. (1990).

The water intake ranged from 11.65 to 12.96 liter /day, and these results show that the amount of water consumed has no effect on the quantities of feed intake by the camels in this study. Hermas (1990) found that the average daily consumption of water per head of camels through the seasons of the year was around 23 liters in the spring, 55 liters in summer and 40 liters in autumn and 16 liters in winter. These results are disagreement with the results of this study which ranged from 11.65 to 12.96 liters /day and this difference may be due to the quality of breed, pasture, type of diet or feeding system.

The Majaheem breed had the best digestibility of dry matter and crude protein, crude fat and soluble carbohydrates compared to other breeds, but Suffr breed recorded the best digestibility

coefficience of crude fiber compared to other breeds. El-Ashry and Sooud (1983) reported that the digestibility of dry matter, crude fiber and nitrogen free extract (NFE) of the camel is more efficient than sheep when fed a low concentration of energy in the diet. The present results demonstrated that there is an effect on feed digestibility. The Majahim had the highest average daily gain, feed conversion and digestibility, followed by Wodoh, Suffr and Sho'l and thus have the ability to utilize the protein and energy food, which would increase the growth and carcass weight and meat quality.

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11. Evaluation of 39 Camelid Microsatellite Markers in Various Breeds of the Dromedary Camel

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Introduction

Camelus dromedarius, often referred to as Arabian camel, is an important species especially in the Arabian Peninsula. In addition to distinct differences among camel species, there are also clear phenotypic variations among breeds or within breeds. They are classified according to color, geographic area, habitat and function. They vary in size and color. Color is the most common phenotypic characteristic used to classify camel breeds (Andersen, 1996).

One particular useful tool for studying genetic traits is molecular markers. Availability of markers would facilitate the precise mapping of desirous or deleterious trait within a family; this would ultimately result in the discovery of genes responsible for these traits. Furthermore, molecular markers can be used for studying relationship within population. So far about 110 dinucleotide markers have been identified from new and old world camelid species (Mate *et al.*, 2005; Penedo, 1999a & b; Sarno, 2000) of which only eight microsatellite markers have been isolated from dromedary camels (Mariasegaram *et al.*, 2002). In this study we investigated the usefulness of 39 of these microsatellite markers for breed identification in dromedaries.

Material and Methods

All samples were collected from different camel farms in Dubai, United Arab Emirates and from other Gulf countries. A total of 584 animals of different Arabian camel breeds (Mahali, Omani, Saudi, Sudanese, Moroccan, Beauty camel and Muhajan) were included in this study. High molecular weight intact genomic DNA was isolated using the Nucleon Blood and Cell Culture DNA extraction kit (Tepnel Life Sciences PLC, UK) from camels' whole blood, collected in EDTA tubes. The quantity of DNA was adjusted to 50-100ng/ µl to be used for PCR.

PCR amplifications forthe 39 markers used were performed in six different cocktails. The forward primers were labelled with VIC, FAM, NED or PET (Applied Biosystems USA).

The amplified fragments were analysed on ABI 3730 XL. Genotypes of individual animals were scored using the GeneMapper software version 4.1.

Preliminary genetic analysis was performed using the GenePop software (Curtin University, Australia) and Cervus software (Field Genetics, UK). Total number of alleles per locus, allele frequency per locus, observed and expected heterozygosity and Polymorphic Information Content (PIC) value for each locus was calculated and compared across breeds.

Results

All 39 microsatellite loci were amplified successfully in the 7 breeds of Arabian camel; showing a total of 373 alleles (range 1- 27, mean 9.56). Two loci named LGU79 and LGU83 were found to be monomorphic.The H_e (expected heterozygosity) value ranged between 0.52-0.58 whereas the H_o (observed heterozygosity) values were found to be in the range of 0.48-0.56 with Omani population on the lowest side and Moroccan population on the highest extreme. The average PIC values for the 39 loci for each breed ranged between 0.458-0.518. Certain alleles seem to be specific for certain breeds only but these alleles cannot be used as diagnostic alleles for that breed. We cannot exclude the possibility of these private alleles existing in other breeds as the sample size for certain breeds was very small (e.g. 10 samples for Moroccan breed). The reason for having a small sample size is the difficulty in obtaining samples from pure breeds. There are certain alleles that have been

found in subpopulations of Sudanese (Anafi population), and appeared only in local, and Muhajan that have been cross bred with Anafi.

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12. Genetic Diversity and Relationships of Indigenous Saudi Arabia Camel *Camelus dromedarius* Populations

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Introduction

The one humped dromedary (*Camelus dromedaries)* and two humped Bactrian (*C. bactrianus)* camels are the largest mammalian species, which are adapted to the desert with its environment of high temperature and extreme desiccation. The camel provides humanity with a range of products and services that make them unique livestock animals adapted for food and agricultural production under harsh semi-desert environments (Kohler-Rollefson, 1993). The dromedary (also called Arabian camel) is one of the best-known members of the Camelidae. Arabian camel played a major role in the daily life and culture of earlier Arab people in the Arabian Peninsula. It was used as a mean of transport and its meat and milk as source of food. They are called "the Gift of God" and "the Ships of the Desert" appreciations to their capability to withstand in arid environment (Sweet, 1965). Today, people in the Arabian Peninsula still drink camel milk and eat camel meat. In recent years, in the Arabian Peninsula, the interest in camels has again increased in racing and beauty contests. Camel racing is an enormous industry in the Gulf countries, with many camels worth in excess of SR 20 million each. The total population of dromedary is estimated to be around 1.6 million camel within the Arabian Peninsula (Al-Eknah, 2008).

Camels in Saudi Arabia

The two-humped camels geographic distribution span the cold desert regions of the southern Mongolia and northwestern China to central Kazakhstan where they withstand snow and well below zero degree Celsius temperatures. They are no established two-humped camel population within Saudi Arabia. While the dromedaries distribution includes the subtropical dry zones of western Asia, northwest India and North Africa, being poorly adapted to humidity and low temperatures (Kohler-Rollefson, 1993). There are about 1 million feral dromedaries in Australia following introduction in the 19th century (Spencer and Woolnough, 2010). Dromedaries were also introduced into parts of the United States of America, Central America, the Caribbean, southern Africa and Europe (Al-Eknah, 2008). In Saudi Arabia there are nine dromedary populations recognized as true populations (no crossbreed was identified): Almagaheem, Almagatter, Alsufur, Alshual, Alhurra, Alshahlia, Alhadana, Alawadi,and Alawarik (Al-Eknah *et al.,* 1997, unpublished). They show difference in morphology (hair structure, colour and body conformation), production traits (meat, milk and sports performances) and adaptive traits (e.g. climate and diseases).

There is no clear classification of Saudi Arabia camel populations with ecological, morphological and utilities criteria generally mixed. Kohler-Rollefson, (1993), divides dromedaries into mountain and plain camels, with the first category subdivided into baggage and riding camels and the latter category subdivided into desert and riverine camels. Al-Eknah *et al.* (1997) divides Saudi Arabian indigenous camel populations into two distinct utility groups; racing and productions camels. The latter group further subdivided into desert, beach and hill camels. Within group and subgroups populations are separated based on morphological criteria or simply on the community owing them. In fact, the naming of the dromedary populations often reflects the locality or country where the camel populations are raised, the Arabs and Bedouin tribes who breed them or simply the animals' colour. It remains largely unknown if these populations are genetically separated from each other.

Molecular Genetics Studies in Saudi Dromedary

In contrast with many other domestic animals, including some Camelid species, there has been no in-depth study on the genetic history of the domestic Saudi Arabian camel populations. Genetic diversity studies in dromedaries have been performed in some countries (Kenya, South

Africa, India, the Caribbean and Australia), using microsatellite markers (Mburu *et al.,* 2003, Nolte, 2005, Mehta and Sahani, 2007, Spencer and Woolnough, 2010). Previous genetic studies in Saudi Arabian dromedaries are limited to a restricted small number of samples, often from a single population or a specific region. For example, Mburu *et al.,*(2003) included 22 camel samples from Saudi Arabia as reference population in their study of Kenyan dromedary genetic diversity. Al-Swailem *et al*. (2007 and 2009) used RAPD and microsatellite markers and assess the usefulness of these markers in paternity testing in three camel populations of central Saudi Arabia.

In the above context, a comprehensive classification of Saudi Arabian camel populations including genetic data will be particularly welcome providing important baselines information for the future management of the domestic species. For the purpose of this study we used the classification of Al-Eknah *et al.* (1997) as reference classification. The present study will therefore aimed to study Saudi Arabian camel populations using microsatellite marker to address questions about their genetic relationship and diversity.

Materials and Methods

Field trips over the raising areas of camel populations in Saudi Arabia have been achieved with support and help of Agriculture and/or Camel Research Centers. The localization of the sampling was based on a previous survey study (Al-Eknah *et al.,* 1997) which indicates the geographic distribution of the indigenous camel populations. A total of 455 samples were collected from unrelated (first and second degree relatives, following the interview of the owners) representing the common camel types in Saudi Arabia.

The DNA extraction procedure from filter papers was optimized with the following protocol modified from Smith and Burgoyne (2004) providing the best result.

Nineteen microsatellite loci have selected from recommended list of The Food and Agricultural Organization (FAO) and the International Society for Animal Genetics (ISAG) livestock diversity committee.

The nineteen markers were initially tested on 24 (unrelated) camels from one population. Gradient PCR (55-65 C°) were performed to test suitable annealing temperature for each marker. PCR is carrying out using the Qiagen Multiplex PCR kit PCR reactions. A volume of 1 µl of 1:10 water diluted PCR product was mixed with loading mix, containing Formamide (Applied Biosystems) and Rox 500 size standard (Applied Biosystems), and then denatured for 3 min at $95^{O}C$ and analyzed in a Genetic Analyzer ABI 3730 DNA sequence (Applied Biosystems).

Results

The result reveals three genetically separated groups of dromedary in Saudi Arabia with distinct, although likely overlapping, geographic distribution in the Southern west region (Alawarik, Alawadi, Alhadana and Alshahlia), East region (Almagaheem and Alshual) and Northern part (Almagatter, Alsufur, and Alhurra) of the Kingdom of Saudi Arabia respectively. The results also highlight that the Alawarik and Alawadi camel populations are genetically distinct from the other camel populations. Phylo-genetic analysis of Saudi and some out-group camel populations from Africa and Asia indicates that most of the genetic diversity of dromedary camels occurs within the Saudi Arabian camel population (Almathen *et. al,.* unpublished). It also supports Saudi Arabia is a likely centre of origin for the domestic dromedary camel. The results obtained will provide evolutionary insights on the history and local adaptation of Saudi Arabian dromedary and contribute to the design of breeding strategies for the conservation of dromedary genetic diversity and the improvement of their productivities.

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Physiology
Biochemistry
Pharmacology
and Immunology

13. Major Proteins and Enzyme Gelatinoletic Activities in Camel Seminal Plasma

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Introduction

Numerous proteins including proteolytic enzymes are found in mammalian sperm. The role of these proteins may be related to their interaction with sperm plasma membranes to maintain optimal conditions during storage and the recognition, binding and penetration of ovum (Morton, 1977). Most proteinases described for seminal plasma are serine proteases and metalloproteases (Cesari *et al.*, 2010). Seminal plasma proteolytic enzymes are involved in coagulation and liquefaction of human sperm (Matsuda *et al.*, 1994).

Studies on camel semen are relatively scarce but it is known that ejaculated semen is very viscous, which needs to be liquefied before its evaluation (Wani *et al.*, 2008). The objective of this project was to study major proteins and to identify proteolytic enzymes in camel seminal plasma. The proteolytic pattern of seminal plasma was compared with that of bovine bull.

Materials and Methods

Semen samples were obtained from 2 mature Maghrabi camels (*Camelus dromedarius*) and 2 bullocks (one Schuitz and one Holstein breeds). Semen was collected using an artificial vagina in January-March period. Camel and bovine bulls were fertile and ejaculates were well characterized. Viscosity of sperm varied between very viscose to slightly viscose in camels and viscose in bovine bulls. After collection, seminal plasma was obtained by two-step centrifugation, the first at 1000 g for 10 min to eliminate spermatozoids and the second at 8000 g for 15 min to eliminate debris. Protein concentration of seminal plasma was determined by Bradford method. SDS-PAGE was performed according to Leammli (1970) to characterize major proteins. Gelatinolytic activity was assessed in 10% polyacrylamide gels containing 0.1% gelatin. After electrophoresis, the gels were washed in Tris-HCl (pH 7.5) containing 1% Triton X-100 and incubated at 37°C for 24 h in the same buffer with 200 mM NaCl and 5 mM $CaCl_2$ or 5 mM EDTA. We assumed that stimulation by Ca^{2+} and inhibition by EDTA indicate the presence of metalloproteases. Proteins were stained with Coomassie Blue. Areas of proteolysis appeared as clear zones against a blue background. Data are presented as mean ± S.E.M.

Results and Discussion

Total seminal plasma protein concentration was 2.0 ± 0.2 g/L in camels and 44.7 ± 1.6 g/L in bovine bulls. Agarwal *et al.* (2005) reported a concentration value equal to 9.2 ± 1.1 g/L in dromedary camels. Mosafari *et al.* (2005) found 22.0 ± 1.0 g/Lin Bactrian species.

In camels as well in bovine bulls four major proteins were visualized in seminal plasma. These major proteins have been found in bovine, human, goat and in many other mammals. However, the molecular weights of these proteins differ according to species. Camel proteins had 10, 15, 18 and 30 kDa molecular weights. The major proteins in bovine seminal plasma represent a family named Bovine Seminal Plasma (Manjunath and Sairam, 1987). They are designated BSP-A1, BSP-A2, BSP-A3 and BSP-30 kDa. Four bands (49.7, 33.2, 26.4, and 19.5 kDa) are found in dogs in which the majority (85%) have molecular weights below 17 kDa, with the 15.6 kDa in high concentrations (de Souza *et al.*, 2007). Contrary to that in bulls, proteins in camel seminal plasma are affected by hot temperature, the 30 kDa protein disappeared after boiling.

Gelatin zymography of seminal plasma revealed numerous distinct proteases ranging from 25 to 72 kDa. In camels, two gelatinolytic band groups could be considered. The first group included proteases with molecular weight less than 54 kDa and the second one regrouped bands higher than 54 kDa. Proteases with low molecular weights (47, 41 and 36 kDa) were more active than the high molecular weight protease (72 kDa). Boiling cleaved the band of 72 to 60 kDa and the band of 36 to 32 kDa. On the other hand, proteases with low molecular weights were more important in viscose sperm. Only 36 kDa band was observed in slightly viscose sperm but 47, 41 and 36 kDa bands appeared in plasma of viscose sperm.

Besides differences in molecular weight, proteolytic enzymes could also be distinguished by inhibition by EDTA. It is known that the addition of EDTA both in the absence or presence of calcium ions resulted in profiles of gelatinolytic activities similar to these obtained without any additives in development solution. All the calcium-activated and EDTA-inhibited bands are metalloproteases. In camels, inhibited enzyme had molecular weight of 72 kDa. However, at least three bands of 47, 55 and 61 kDa metalloproteases were observed in bovine seminal plasma. Gelatinolytic profile of camel seminal plasma metalloproteases is different to matrix-metalloproteases activities observed in epididymal fluid of many other domestic mammals (Métayer *et al*., 2002). Considering the independence of serine proteases from Ca^{2+}, it could be observed that there is no serine-like proteases with molecular weights >54 kDa.

It was concluded that camel seminal plasma is characterized by a low protein concentration, four major proteins and several gelatinoletic activities proteases, including metalloproteases and serine-proteases.

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14. Peripheral Concentrations of Glucose, Metabolic and Steroid Hormones Relative to Birth Date, Live Body Weight and Average Daily Gain in Prepubertal Shami Female Dromedaries

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Introduction

Nutritional status, season of birth, and breed of camel can affect the onset of puberty (Tibary *et al*., 2005). The reproductive performance of animals is related positively to the animal's body- fat mass (Kennedy, 1953). Insulin-like growth factor-I (Velazqueza, *et al.,* 2008) and leptin (Moschos *et al.*, 2002) have been found to act as a metabolic signal that allows reproductive events to occur. A significant positive correlation between body condition score and plasma leptin levels is found (Delavaud *et al.*, 2000). Prolactine has actions on the whole animal with high concentrations from birth onwards in cows (Schams and Reonhardt, 1974). Camels can maintain blood concentrations of glucose, but metabolic body size affects both its entry and utilization rates (Chandrasena *et al.,* 1979). Levels of estrogen and progesterone during the dromedary cycle in mature, but not in immature females have been studied widely (Agarwal *et al.,* 1991).This paper aimed to determine the peripheral concentration of glucose, IGF-1, leptin, prolactin, estrogen and progesterone relative to age, live body weight and average daily gain during the growing prepubertal stage in Shami female dromedaries.

Materials and Methods

Ten post weaningShami female dromedaries (27 weeks of age) with an average body weight at birth of 28 ± 2 kg and born between January to May raised at Shami Camel Research Station in Syria were used. Body weight was measured and blood samples were collected weekly from the jugular vein for 6 months. Serum was separated by centrifugation ($2,300 \times g$ for 25 min; 4°C) and stored at −20°C for later analysis. Glucose was determined using a colorimetric glucose oxidase kit (Kolath *et al*.,2006) and validated for camel serum. Serum concentrations of prolactin, leptin, insulin-like growth factor-I (IGF-1), estradiol-17b, and progesterone were all assayed using bovine radioimmunoassay procedures (Scharf *et al*., 2010) and validated for camel serum. The glucose and protein hormone assays were analyzed in triplicate and the steroid hormone assays were analyzed in duplicate. Inter- and intra-assay CV's were less than 10%. Serial dilutions of pooled aliquots of camel serum were linear (log/log it transformation; $R^2 > 0.98$) and parallel to both standard curve concentrations and serial dilutions of pooled aliquots of bovine serum. Dromedary heifers were classified according to their birth date, BD into three groups (G1, n=3 from Jan. to Feb., (G2, n=3 : born in March and G3,n=3 : Apr. to May); to their live body weight, BW at last blood collection into two groups: (G1, BW is < 210 kg, n=4 and G2 is > 210 kg, n=6); or according to their average daily gain ,ADG (G1, n=4 ADG is < 450g and G2. n =6 is > 450g). Variations in the plasma concentrations of studied parameters within and between groups and the effect of age, BD, BW and ADG were tested and assessed by analysis of variance using the general linear model, repeated measurements procedures of the SAS.

Results and Discussion

Analysis of variance indicated no significant effect for BD, BW or ADG on the glucose, leptin prolactine or progesterone levels, but there were significant effects ($p<0.05$) for ADG and BW on estradiol-17 β and IGF-1, levels, respectively. Animals in G3 showed greatest values of estrogen and dromedary heifers having higher BW (G2) showed greater values of IGF-1.

Results indicated that plasma glucose concentrations (188 ± 4.1 mg/dl) were higher than those (100-138mg/dl) reported in mature dromedary females (Kumar and Banerjee, 1962; Al-Ali *et al*., 1988) and in true ruminants (45-55 mg/dl; Ballard *et al*., 1969). Concentrations of IGF-1 in Shami dromedary heifers (222 ± 6.27) exceeded largely those in growing heifers of different beef breeds (94 to 129 ng/ml; Jones *et al*., 1991) and almost similar to those of ewe lambs (249 ± 8 ng/ml; Roberts *et*

al.,1990). Leptin concentration (13.38 ± 0.16 ng/ml) was three times higher than in mature dromedary females (Delavaud *et al*., 2004) much higher than in cattle (6 -7 ng/ml; Chilliard *et al*., 2005,). It seems, this high level of leptin, in prepubertal dromedary heifers might be involved in accelerating the onset of puberty. Results showed a considerable secretion of prolactine (6.07 ± 0.32 ng/ml) which was two folds higher than in prepubertal gilts (2.3 to 6.6 ng/ml; Diekman *et al*., 1983). Varying concentrations of estradiol from 0.01 to 3.12 pg/ml probably reflects to some degree the presence of ovarian activities in prepubertal Shami dromedary heifers. This agrees with results reported for Turkmenistan dromedary heifers aged 8 to 12 months and showing normal follicular dynamics (Abdunazarov, 1970) and with those reported in mature Chaambi dromedary females(< 0.5 pg/ml) during the onset of the breeding season in Algeria (Adamou *et al*., 2009). Serum progesterone concentrations (0.07 to 0.1 ng/ml) were low through the prepubertal period in Shami dromedary. This is in agreement, but with lower values reported for ewe lambs (< 0.2 ng/m; Ryan *et al*., 1997), for prepubertal cattle heifers (0.26 ± 0.08 ng/ml; Gazal and Anderson, 1995). However, the source of progesterone in this study needs to be investigated.

Conclusion

Such information might be helpful for specialists to search for the role of these hormones in the reproductive function during the early life of this species and to develop programs to reduce the period of onset of puberty and increase the reproductive performance of dromedary camels which have a tremendous socio-economic important role in the dry area.

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15. Serum Protein Capillary Electrophoretic Patterns in Camels (*Camelus dromedarius*): Influence of Age and Sex

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Introduction

Plasma proteins are known to comprise about 6-7 g/dl (Eckersall, 2008). Functionally, plasma proteins are involved in nutrition, maintenance of osmotic pressure, buffering acid-base balance, transport of smaller ions and molecules, haemostasis and protective effect of the immune proteins (Eckersall, 2008). Many of these plasma protein change markedly in diseases (Abate *et al*., 2000; Rasouli *et al*., 2005) and with age (Keay and Doxy, 1982; Chaudhary *et al*., 2003).

Capillary electrophoresis of serum proteins (CE) is an established and effective method which has been used as a screening tool for the clinical diagnosis of many diseases in humans (Jellum *et al*., 1991; Gay-Bellile *et al*., 2003) and animals (Camacho *et al*., 2005). Normal serum proteins electrophoretic patterns are composed of five fractions, albumin, α_1-globulin, α_2 globulin, β-globulin and γ- globulin (Eckersall, 2008). Therefore, the clinical interpretation of CEP is based on the variation in the content of one or more of these five major fractions. However, species differences between the animals have been observed by Keay and Doxy, (1982). Therefore, the aim of the study was to validate the use of CE in camels and to determine the normal serum protein capillary electrophoretic pattern in relation to the age and sex.

Materials and Methods

Blood samples were collected form fourteen healthy young camels (7 males and 7 females, age: 3-5 months) and 22 adult camels (12 male and 10 female, age: 5-8 years) by using plastic syringes (7.5 ml, Pirmvetta®, Laboratory Technique, GmbH, Germany). The samples were centrifuged and the serum was collected in sterile containers and frozen at -20°C. The fractionation of serum proteins was determined by using a capillary electrophoresis technique by with a biochemical analyser (Roche Hitachi Modular, Roche).

Statistical analysis was performed using SPSS for Windows Version 17.0. The distribution of the individual data was determined by using a One-Sample Kolmogorov-Smimow adjustment test. The statistical measurements of serum total protein fractions were estimated by using descriptive statistics procedures of the same programme. ANOVA tests (Levine's Test and Post Hoc Test) were used to assess the possible significant differences between the age groups. The mean difference was considered significant at $P \leq 0.05$.

Results

Figure 1 shows the normal pattern of CE in dromedary camels. The pattern of CE identified one albumin, two α- globulin (α_1 and α_2), one β-globulin and one γ-globulin fractions.

Figure 1. Serum protein capillary electrophoresis pattern of healthy camels (*Camelus dromedarius*) of various ages and sex (n = 36).
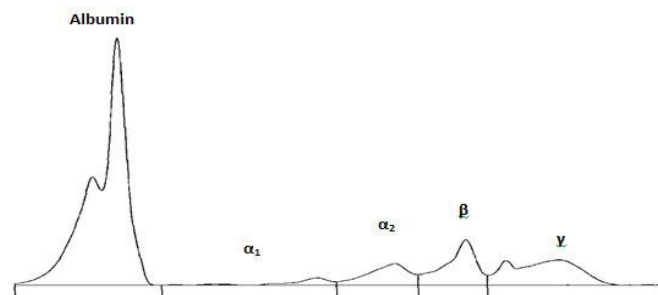
The mean values of serum total protein and CE fractions are shown in Table 1. The higher significant (P<0.05) mean value for serum- [Protein] of 63.7±6.6 g/l (reference range= 51-74 g/l) was observed in the female camels compared to the other age groups. Adult male camels showed a highly significant (P<0.0001) higher percentage of albumin fraction (60%) compared to the other age groups.

α_1 and α_2 globulin fractions showed a significant (P<0.01) higher mean values in young camels compared to the other groups (3.5% and 8.5%, respectively). β-globulin fraction was not affected significantly by the age. Lactating female camels showed a significant (P<0.01) higher mean value of γ- globulin fraction (26%) compared to the other age groups. The lowest significant (P<0.001) A/G ratio (1%) was observed in lactating females.Sex had no significant effect on serum protein fraction.

Discussion

The main finding of the present study is that CE has been applied to the serum of dromedary camels. CE produced five peaks comprising one albumin, α_1 and α_2, β and γ- globulins fractions (Figure 1). However, in camels Chaudhary *et al*., (2003) have reported that serum protein electrophoresis on agarose gel produced six peaks comprising one albumin, α_1 and α_2, β_1 and β_2 and γ- globulin fractions. The variation in the serum electrophoresis pattern between the present study and the study conducted by Chaudhary *et al*., (2003) may be due to differences in methodologies used.

The reference range of serum- [Protein] obtained in the present study for adult camels (51-74 g/l) was similar to the values reported previously for adult racing camels (Abdalla *et al*., 1988, 59-64 g/l; Mohamed and Hussein 1999, 53-78 g/l). However, the mentioned range was lower than that reported by Bogin 2000 (63-88 g/l). The mean value reported for young camels (54.8±4.0 g/l) was within the reference range reported for young camels at the age of 1 year old (Haroun 1994, 49-85 mmol/l). The variations in the concentration of serum total protein can be explained by the variation in the nutritional status of the animals. In lactating female camels, the higher mean value of serum-[Protein] (63.7 g/l) can be due to the higher concentration of γ- globulins observed (26%=16.8 g/l) (Table 1).

Albumin represented the main fraction of serum proteins determined by CE in all groups (50-60%, Table 1). α_1 and α_2, β and γ- globulin fractions represented about 3-4%, 9-10%, 10-12% and 18-26%, respectively (Table 1). These findings are higher than those reported for adult and young camels (Chaudhary *et al*., 2003). Furthermore, the present results indicate that there was a significant difference between the adult male, female and young camels in the fraction of albumin, α_1, α_2, and γ-globulin. The variation in these values can be considered as an age-dependent relationship between the groups.

Table 1 Serum protein capillary electrophoresis pattern of healthy camels (*Camelus dromedarius*) of various ages

Parameter	Age group					
	Young male and female camels (n = 14. 3-5 m)		Lactating females (n = 10. 5-8 yrs)		Adult males (n = 12. 5-8 yrs)	
	m± s	[1]reference range	m± s	[1]reference range	m± s	[1]reference range
Serum-[Protein] (g/l)	54.8±4.0[a]	50-62	63.7±6.6[b]	51-74	57.7±4.3[a]	51-65
Albumin fraction (%)	58±4.7[a]	52-67	50±4.9[b]	39-54	60±2.2[a]	57-63
α_1- globulin fraction (%)	3.5±0.5[b]	2.6-4.4	2.6±0.4[a]	1.9-3.3	2.7±0.6[a]	1.8-3.9
α_2- globulin fraction (%)	10±1[a]	8.5-12.6	9.4±1[b]	7.8-10.9	8.6±1[b]	7.5-11
β-globulin fraction (%)	10.4±0.8[a]	9-12	11.7±1.7[a]	8.5-14	10.9±0.7[a]	9.9-12
γ-globulin fraction (%)	17.7±5.2[a]	9-25	26.3±5.2[b]	23-39.5	17.7±1.7[a]	15.2-21
A/G ratio	1.4±0.3[a]	1.1-2	1±0.2[b]	0.6-1.2	1.5±0.1[a]	1.3-1.7

m± s×1.96 indicated the lower and the upper limits, Brackets ([ ]) donate concentration, m= months, yrs= years
[a, b] Means within the same row bearing different superscripts are significantly different at p≤0.05.

Conclusion

The present results indicate that variations in the serum electrophoresis pattern of the camels between the present study and those reported previously in the literature may be due to the age factor. The physiological and the nutritional status of the animals may play a significant role in these variations.

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16. A Survey on Antimicrobials Utilized in Camel Practice by Private Veterinary Practitioners in Oman

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Introduction

Antimicrobial therapy ideally is determined by isolation of the offending organisms and determination of their antibiotic susceptibility pattern. This information is usually not available in the field, where veterinarians often make treatment decisions based on the likelihood of an organism being assumed to be the etiologic agent after considering the clinical signs of the patient. In addition, antimicrobials are often administered by the camel owners in Oman. This practice raises important concerns relative to the possibility of inducing antimicrobial resistance. Compounding this reality is that the dosages for camels are often extrapolated from other large animals and this assumption might not be accurate (Ali *et al*., 1996). The present study aimed to develop a data base of antimicrobial agents that are commonly used by private veterinarians in Oman working with camels.

Materials and Methods

A questionnaire was presented to clinical veterinarians, listing forty antimicrobials, and asking the number of patients that they treat, their preferred route of antimicrobial administration both to adult patients and to camel calves and the percentage of cases they were able to personally follow-up. The survey was pretested with two veterinarians to check the appropriateness of the language utilized in the survey. They took approximately 30 minutes to complete the survey. They did not have any difficulty in understanding the questions, which ruled out the need for a bilingual questionnaire (English/Arabic). All the participating veterinarians were briefed about the survey on the first visit and the questionnaires were collected on the next visit to the practice. A total of 23 questionnaires were distributed among the private vets of different regions in Oman such as Ash Sharqiyah (n=9), Al Batinah (n=12), Al Dakhliyah (n=1) and Al Buraimi (n=1). Responses were analyzed and the results were shown in terms of the most preferred antimicrobials, and the number of patients that they treat on an average per month. The questionnaire also asked why follow-ups were not conducted and listed possible reasons (Table 2). The survey responses were analyzed in Microsoft Excel[R]2010, using general tools as filtering and percentile to check the frequency preference of the antimicrobials listed in the questionnaire.

Results

From a total of twenty three veterinarians, who received the questionnaire, there were twenty respondents. One veterinarian declined to participate and two had not filled in their questionnaire in time for their results to be analyzed and include in these preliminary results. The results of this study are summarized in the Table 1.

Table 1: Frequency of use of antimicrobial agents

Name of the antimicrobial	Frequency of Use (%)
Oxy tetra cycline-Long acting-200mg/ml	98.75
Tylosin	98.75
Trimethoprim /Sulfonamide combination	87.5
Strepto penicillin	82.5
Enrofloxacin	81.25
Amoxycillin (Long acting) LA	78.75
Sulphadimidine	75
Sulfamethazine	73.75
Sulfadiazine	67.5
Penicillin procaine	63.75
Sulfaguanidine	63.75
Benzyl penicillin (Penicillin G)	62.5
Ampicillin	61.25
Neomycin	56.25
Amoxycillin + Cloxacillin	53.75

Benzathine penicillin	52.5
Cefalexin/Cephalexin	51.25
Oxy tetra cycline-Plain-50mg/ml	48.75
Norfloxacin	47.5
Ampicillin + Cloxacillin	43.75
Lincomycin	36.25
Doxycycline	30
Chloramphenicol	28.75
Kanamycin	28.75
Colistin	27.5
Erythromycin	26.25
Gentamicin	26.25
Spectinomycin	21.25
Bacitracin	17.5
Fusidic acid	13.75
Novobiocin	12.5
Tilcomicin	10
Ceftiofur	8.75
Florphenicol	8.75
Amikacin	7.5
Cefazolin	6.25
Cefuroxime	3.75
Monensin	3.75
Rifampicin	1.25
Lasolacid	1.25

Ten veterinarians treat less than 50 camel patients a month, six veterinarians treat between 50-100 camel patients and four veterinarians treat between 100-200 camel patients per month. On an average 65% of the clinical situations required antimicrobial treatment. From these treatments they were able to conduct follow-ups on 71% of the patients. In Table 2 reasons for not being able to conduct follow-up treatments are shown. The preferred route of antimicrobial administration in adult camels was the intravenous route (92.5%) and the *per os* route in camel calf patients (86.25%).

Table 2: Reasons for not following-up patients treated with antimicrobial agents

Reasons	Yes	No	Not answered	Percentage of their agreement with the reasons cited
Camel herds are often remotely located from your clinic	10	9	1	50
Persisting owner's demand, that they would take care of the follow-up	13	6	1	65
Owner's affordability in bearing your visit charges for repeat visit days	9	9	2	45
Non availability of Para veterinary professionals like Veterinarian assistants and technologists	9	8	3	45

Discussion

To the best of our knowledge this is the first study in Oman undertaken to ascertain information from field veterinarians in regards to the use of antimicrobials commonly encountered in their camel practices. The results showed that three out of five preferred antimicrobials belonging to the older antimicrobial groups such as tetracyclines, streptomycin, penicillins, and sulphonamides. The least preferred antimicrobials belonged to the newer generation of cephalosporins, aminoglycosides and thiopenicols. As there is an increase in antimicrobial resistance worldwide against the older antimicrobial agents, such as tetracyclines, streptomycin, penicillins, sulphonamides, and less prevalent against the newer cephalosporins, quinolones, and macrolides (Morley, 2005) it is a fair assumption to question the effectiveness of camel treatments and the possibility of diminishing

effectiveness of these antimicrobial agents against bacterial pathogens. Compounding the problems associated with the use of antimicrobial agents is the fact that many camel owners administer treatments to their animals and that inappropriate doses may be used, as the amounts given are often merely extrapolated from other large animals and this assumption might not be accurate. It would be highly desirable to have regional diagnostic laboratories where bacterial diagnostics could be carried out and susceptibility tests performed so that emerging trends of resistance could be monitored and clinical veterinarians could be advised on appropriate antimicrobial treatments to administer to their camel patients.

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17. Preventive and Curative Ethnoveterinary Plant Remedies Applied by the Rendille and Gabra Camel Keepers of Marsabit District, Northern Kenya

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The need for increasing the options for prophylactic and therapeutic inputs and services against livestock diseases/ailments among the resource poor camel keeping pastoral communities of Marsabit District is evident. Due to the vastness and the remote residence of camel keepers, access to modern veterinary inputs and services is limited or totally non-existened. This is because camels in Kenya are reared far from any conventional veterinary delivery systems. In addition not many veterinarians or even veterinary para-professions are willing to set up practices in ASAL regions of the country due to the high operational costs. On the other hand the Government veterinary outreach is also inadequate and where it exists, the personnel are usually poorly facilitated to offer meaningful services to the camel keeper. As a result of this inherent benign animal health delivery challenges, Rendille and camel keepers of Northern Kenya have developed an elaborate traditional camel health care and healing system that has served them well. However continued use of flora based ethno-veterinary practices is threatened by the gradual loss in biodiversity. This is a result of environmental degradation occasioned by a combination of many factors that include: increasing sedentarization and changing pastoral life styles, over grazing, impact of climate change and global warming among others.

The study involved identification of livestock traditional healers and assessment of the level of existing veterinary knowledge (EVK) and practices; collection, documentation and botanical identification of the plant species and materials that were considered usable for managing livestock diseases by the study communities and screening of all the medicinal plants cited for antimicrobial activity.

In total, 72 plant species in 34 families were inventorized as medicinal plants applied by the two communities in the prevention and curation of more than 20 livestock diseases. Some non-plant based folk practices were also documented. Burseraceae, Caparidaceae, Euphorbiaceae, Mimosoideae and Solanaceae were the five most common plant families. They contained 22 out of the 72 medicinal plant species identified. Most of the diseases/ailments treated using the plant remedies could be visualized into several broad but distinct categories. These included: internal disorders; external injuries or ailments; eye infections; infertility and retained afterbirth (RAB) and mineral deficiency. Sensitivity tests revealed that some of the herbal plants in use by the two communities like *Terminalia brownii* have very high antibacterial activity. This was demonstrated by use of Muller-Hinton-Agar (MHA) inoculated with *Micrococus lutea* and *Bacillus cereus*, using both the well and disc reservoir methods and utilizing water and ethanol as the solvents. Out of the 36 medicinal plant species available for screening for antibacterial activity, 21 were from Rendille community while 15 were gathered from Gabraland. *Terminaliabrownii* from Rendille region showed the highest activity against *M.lutea,* with an inhibition zone (diameter) of 24.0 mm and 25.0 mm with ethanol and water extracts respectively, using the disc-method. Using the well-method, the same herb showed an inhibition zone of 24.0 mm and 23.0 mm with ethanol and water extracts, respectively. Water extract of *Balanites aegyptiaca* from Gabraland using disc-method gave an inhibition zone of 10.0 mm and 7.0 mm against *Bacilus cereus* and *Micrcoccus lutea* respectively. Under the same extraction method, *Solunum incunum* showed 9.0 mm and 9.0 mm for *B. cereus* and *M. lutea* respectively. *Cucumis dipsaceus* gave the highest inhibition zone (14.0 mm) with *B. cereus* compared to 7.0 mm showed by *M. lutea* after ethanol extraction in well- method while water extraction of *Commiphora flaviflora* gave 10.0 mm and 9.0 mm against *B. cereus* and *M. lutea* respectively.

Based on these findings, it can be concluded that folk veterinary practices among the Rendille and Gabra communities of Marsabit District exist and that some of the medicinal plants used by these ethnic groups contain demonstrable antibacterial activity. It is therefore recommended that, there is a need for further research to carry out clinical trials that would validate the efficacy of these remedies and develop treatment regimes/crude dosing guidelines for the proven remedies. This should be based on locally available gadgets (e.g. how much handful of roots should one boil in how much water to obtain an equivalent of how many 300 mls bottles of *"Coca Cola soda"* to get an effective dose?

18. ncidence of Mastitis in One-Humped Camels (*Camelus dromedarius*) Under Pastoral Management in Semi-Arid North-Eastern Nigeria

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Introduction

Mastitis, an inflammation of the udder, could be a potential source of low milk yield and income in postoral camel herds (Blowey, 1999), especially in nothern Nigeria. The disease can be infectious, caused as a result of physical injury to the mammary gland or contagious caused by microorganisms. The inflammatory responses result in higher blood proteins and white blood cells in the mammary tissues, which passes into the milk. The quality of milk is altered by changes in composition and by increase in somatic cells. Edmonson (2004) reported a low milk calcium and potassium levels in mastitic milk. The initial micro flora of milk reflects microbial contamination during production process. Sub-clinical camel mastitis drastically reduces milk yield. The loss in milk as a consequence of mastitis was ranked high by herdsmen than the loss of calf (Younan *et al.,* 2004). In northern Nigeria, camel milk is extensively consumed by nomads, fresh and soured and sometime mixed with cow milk make cheese (Kalla, *et al.*, 2007). The current study was designed assess the incidence of mastitis in local camel herds under extensive pastoral management system.

Materials and Method

The study was conducted in Azare and Gamawa Local Government Areas of Bauchi State, Nigeria. Bauchi State lies between latitudes $9^o.3^'$ and $12^o.3^'$N and longitudes $8^o.5^'$ and $11^o.0^'$E. It has a human population of 2,826,440 (BSADP, 2006).The State lies within the Sudan Savannah ecological zone. The rainfall in the area ranges between 1000mm and 1300mm/annum. The relative humidity ranges from about 12% in February to about 68% in August. The pastoralists managed their animals extensively.

A total of 100 quarter milk samples were collected from 25 lactating camels at different stages of lactation and analyzed for composition and presence of mastitis causing organisms as described by Younan *et al.* (2000). Camels herds were at three different locations of approximately 50 km apart (Azare, Udubo and Yaba). Antibiotic sensitivity tests were carried out on all the isolates using commercially available antibiotic (Nilsson *et al.,* 1994). The data were analysed for variance and the frequency of mastitis causing isolates, sensitivity test results and staining properties were expressed as percentages.

Results and Discussion

The camel milk used in the study showed a significant difference in percentage milk protein due to location of herd. However, no significant difference was recorded on fat, lactose, moisture and solids not fat (SNF).Table 1 shows the distribution of bacterial isolates in camel milk during the study. *Staphylococcus aureus* was the dominant isolate (24%). Others included: *Klebsiella spp* (16%), *Streptococcus spp* (16%) and *β-haemolytic streptococcus* (12%). *E.coli* was the least frequent isolate (8.0%). A mixture of *Staphylococcus* and *Streptococcus* made up 16% of the isolates.

Table 1. Percentage of isolates of camel milk in the study area**.**

Isolates	Herds			
	A (n=9)	B (n=7)	C (n= 9)	Total (n=25)
Staphylococcus aureus	33.33 (3)	42.86 (3)	0	24.0 (6)
Klebseilla spp	11.11 (1)	42.86 (3)	0	16.0 (4)
Streptococcus spp	11.11 (1)	42.86 (3)	0	16.0 (4)
β-*Haemolytic Streptococcus*	11.11 (1)	14.29 (1)	11.11 (1)	12.0 (3)
Steptococcus feacalis	11.11 (1)	14.29 (1)	11.11 (1)	12.0 (3)
α - *Haemolytic streptococcus*	0	14.29 (1)	22.22 (2)	12.0 (3)
Eschericia coli	0	0	22.22 (2)	8.0 (2)
Klebsiella +Staphylococcus	11.11(1)	22.22(2)	0	12.0(3)
Staphylococcus+ streptococcus	11.11(1)	42.86(3)	0	16.0(4)

No growth	44.4(4)	14.29(4)	22.22(2)	40.0(10)

Figures in parenthesis are the frequencies

Abdel Gader *et al.* (2005) reported *Staphylococcus aureus* to be the main pathogenic bacteria occurring in camel milk The sensitivity and efficacy of various antibiotics on bacterial isolates in camel milk is shown in Table 2. Results indicated that the isolates were sensitive to Gentamycin (100%) then Chloramphenicol (85.7%) Sparflox, Augmentin, Erythromycin and Streptomycin (71% each). Ampicillin had the least effect on the isolates 28.6%. The isolate α-*haemolytic streptococcus* was sensitive to all the antibiotics. However, *Staphylococcus aureus* was sensitive to most of the test antibiotics except Penicillin, Amoxicillin and Streptomycin. Gentamycin like other quinolone class was effective against *Staphylococci* and showed excellent activity against gram negative bacilli (Kalla, *et.al.,* 2008). Most of the organisms were resistant to ampicillin and this agrees with the report of Mekonnen *et.al.* (2005).

Table 2. Percentage Sensitivity of Isolates to test Antibiotics

*Antibiotics	Isolates							% Sensitivity
	1	2	3	4	5	6	7	
Penicillin	R	S	R	S	R	S	R	57.0
Amoxicillin	R	S	R	S	R	S	S	57.0
Ampicillin	S	R	R	R	R	S	R	28.6
Cloxacin	R	S	R	R	S	S	S	57.0
Streptomycin	S	S	R	S	S	S	R	71.0
Chloramphenicol	S	S	S	S	R	S	S	85.7
Erythromycin	S	S	R	S	R	S	S	71.0
Tetracycline	S	R	R	R	S	S	R	42.0
Augmentin	S	R	S	S	R	S	S	71.0
Gentamycin	S	S	S	S	S	S	S	100.0
Spafloxacin	S	R	R	S	S	S	S	71.0

1= *Staphylococcus aureus;,* 2= *Klebsiella spp*; 3= *Streptococcus spp*; 4=β-*haemolytic streptococcus*; 5= *Streptococcus feacalis;*6= α-*haemolytic streptococcus;,* 7= *Escherichia coli*; R= Resistant; S= Sensitive. *Trade names

The staining property indicated that 60% of the total isolates were gram positive cocci in singles, 24% gram positive cocci in chain, 12% gram negative bacilli while the least was gram negative rods (12%). Higher bacterial cell counts were observed in late lactation (216×10^3 cell/ml) compared to early (132.66×10^3cell/ml) and mid (177.56×10^3 cells/ml) lactations.

Conclusion

It was concluded that the microflora isolated from the camel milk in the study is a potential cause of mastitis in dromedary camel. The antibiotics tested (especially gentamycin) had higher potency against the mastitis causing microrganisms.

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19. Pyrethroid (*Lambda cyhalothrin*) Poisoning in Camels

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Introduction

Synthetic pyrethroids are neuropoisons acting on the axons in the peripheral and central nervous systems by interacting with sodium channels in mammals and/or insects (Ruigt *et al.*, 1986, Vijerberg *et al.* 983, Wang *et al.*, 2007). Lambda cyhalothrin is a pyrethroid insecticide used for controlling pest insects in agriculture, public health and in construction and households (European-Commission, 2001, Lawler *et al.*, 2007). Under alkaline conditions it hydrolyses to form cyanohydrin which degrades to form hydrocyanic acid and the corresponding aldehyde (He LM *et al.*, 2008). It is highly toxic for zebrafish, shrim fish, frogs, rats, and bees (Kumar *et al.*, 2007, Ruigt *et al.*1986, Smart, *et al.*, 1982, Verschoyle, *et al.*, 1980).

Studies using laboratory animals showed that Lambda cyhalothrin may induce neurotoxic effects such as staggering gait, muscle tremors and convulsions. (Kumar, *et al.* 2007, Van Den Bercken, *et al.*, 1979, Vijverberg, *et al.*, 1982, 1982 b, 1983).

In humans, the clinical signs of poisoning include irritation of the eyes, irritability, headache, dizziness, nausea, vomiting, diarrhea, excessive salivation, fatigue, muscle twitching, fluids in the lungs, muscle twitching, and seizures (Gu BG, *et al.* 2007). No records were obtained on Lambda Cyhalothrin poisoning in field animals.

Case History

Lambda Cyhalothrin acute poisoning occurred amongst a herd of 132 hungry adult female camels that grazed on *Sesuvium vericusum* plant, (family Aizoaceae) sprayed with this insectside. The morbidity rate was 100%. The mortality rate was very high 81.82%, (Figure 1).

The clinical signs appeared 15-20 minutes after the consumption of the sprayed plant. Death occurred 2-4 hours after the onset of the clinical signs. Due to the lack of oxygen, the brain and heart were the first to be affected. The affected camels showed an increased rate of respiration and pulse rate, muscular tremors, incoordination (Figure 2), staggering gate, excitement, salivation, vomiting (Figure 3), defecation (Figure 4), bloat, blue coloration of the mouth and eyes mucous membranes, recumbency, terminal convulsions and death. At postmortem the venous blood was bright red in color.

Materials and Methods

Sprayed plant, blood and serum, stomach and intestinal contents, liver, kidney, heart, brain and abomasum were collected for toxicological examination. The blood was examined using Vetscan HM 5 Hematology system. The serum was examined using ACE Alera Clinical chemistry System. Small intestine, kidney, liver, heart, brain and abomasum were immediately fixed in formal saline. Later they were embedded in paraffin wax, sectioned and stained with haematoxylin-eosin stain.

Laboratory Findings

The blood showed no significant changes. Serum examination showed significant increase in creatine kinase (CK), y-glutamyltransferase (GGT), glucose, urea, alkaline phosphatase (ALP), triglyceride (TRI), and sodium levels. A significant decrease was seen in uric acid and magnesium levels (Table1). The kidney showed intertubular and glomerular hemorrhages, degeneration (Figure 5, and Figure 6), and peripheral hemorrhages with separation and distension of the renal capsule. The liver (Figure 7) and abomasum (Figure 8), showed severe hemorrhages and cellular degeneration. The heart showed severe hemorrhages and fragmentation and separation of myofibers, due to the hemorrhages, and cellular infiltration (Figure 9). The brain showed diffused liquefaction necrosis which destroyed most of the grey matter (Figure 10). The small intestine showed severe hemorrhagic enteritis (Figure 11). Screening of the stomach and intestinal contents using gas chromtograph and mass spectrophotometer of the stomach and intestinal contents revealed the presence of the ingested Prethroid insecticide.

Treatment

Due to the acuteness of toxicity it was too late to treat an affected animal after the signs were recognized. Only supportive intravenous treatment was given in the form of liters of 2 Linger Lactate and 3 liters of Normal Saline given simultaneously.

Discussion

The significant increase in CK resulted from the muscular dystrophy and myocardial infarction. The significant increase in ALP, GGT, and GLU, and the decrease in Uric Acid and MG indicates liver damage. The increase in Urea and TRI and NA, and the decrease in MG indicates renal failure due to decreased glomerular infiltration rate as a result of renal insufficiency.

Table 1 : Serum biochemical values in 9 poisoned camels

Parameter	CK	ALP	GGT	GLU	UREA	UA	MG	TRI	NA
NORMAL RANGE	26-69.8 U/L	41-103 U/L							114-133 MMOL/L
ANIMAL NO. 1	154	118							178
ANIMAL NO. 2	148	175							186
ANIMAL NO. 3	304.1	215							197
ANIMAL NO. 4	155	187							178
ANIMAL NO. 5	976.4	180							185
ANIMAL NO. 6	3127.6	229							188
ANIMAL NO. 7	558.3	178							177
ANIMAL NO. 8	1328.6	252							179
ANIMAL NO. 9	914	167.4							175

CK: Creatine Kinase ALP: Alkaline Phosphatase GLU: Glucose
GGT: Y-Glutamyltransferase UA: Uric Acid MG:Magnesium
NA: Sodium TRI: Triglyceride

Figure 1.
Very high mortality rate

Figure 2.
Incoordination

Figure 3.
Vomiting

Figure 4
Defecation.
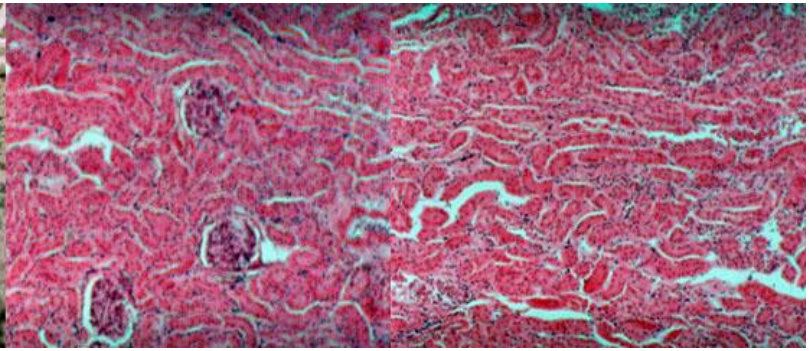
Figure 5.
Kidney: Intertubular and glomerular hemorrhages and degeneration.

Figure 6.
Kidney: Intertubular hemorrhages and degeneration.
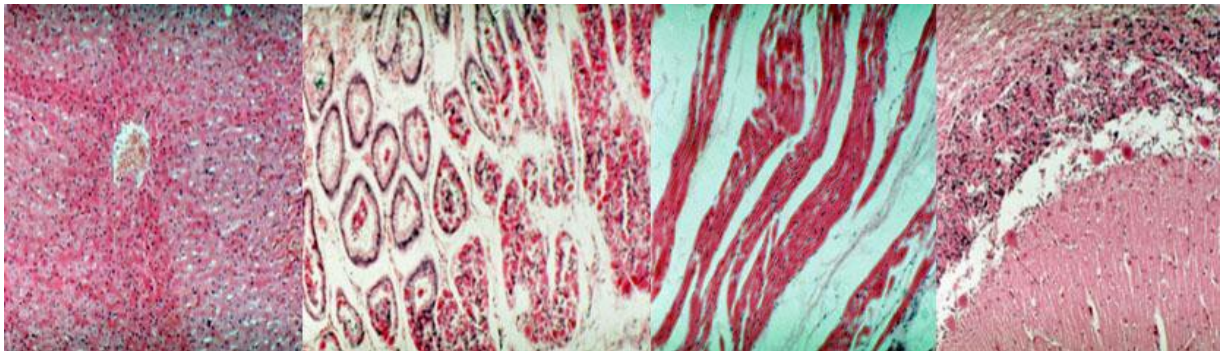
Figure 7. Liver: Severe hemorrhages and cellular degeneration.
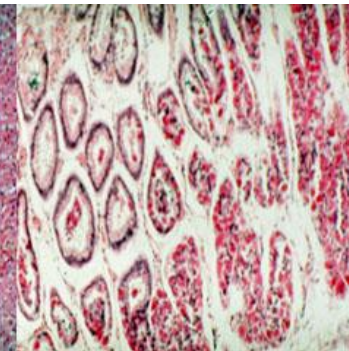
Figure 8. Abomasum: Severe hemorrhages & cellular degeneration
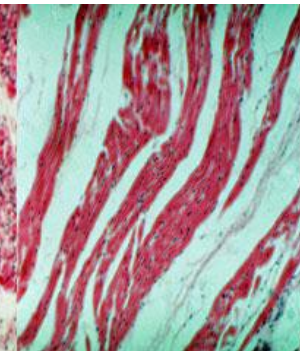
Figure 9. Heart: Severe hemorrhages & fragmentation & separation of myofibers
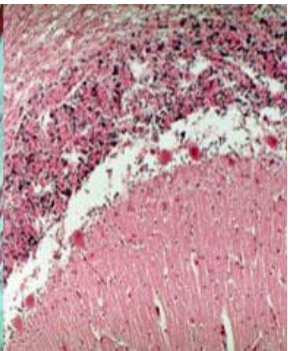
Figure 10. Brain diffused liquefaction necrosis

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20. Search For The Best Adjuvant For Use in Dromedaries

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Introduction

Immunological research of the camel has highlighted the benefits of this animal as a model for pathogenic diseases and as a potent source of antibody production (Abbas and Agab, 2002) including the development of novel antisera, serological testing methods, and even biomarkers (Deffar *et al.*, 2009). Discovered by Ramon *et al*, in the 1920s (Ramon, 1925), adjuvants are compounds that augment or prolong a specific immune response when injected in conjunction with an antigen, without having any antigenic properties themselves (Vogel *et al.*, 1998). At the Central Veterinary Research Laboratory (CVRL) in Dubai, both FCA and FIA had been previously used to enhance antibody production in the dromedary camel. Unfortunately, this use was associated with severe inflammatory reactions at the injection site. However, to maximise antibody production, a camel must be re-inoculated multiple times with up to six booster vaccine doses before a suitable antibody titre can be reached. Subsequently, other adjuvants needed to be tested in camels.

Material and Methods

Seven different adjuvants were used in the trial: oil-based emulsion adjuvants such as Gerbu Vet and Montanide ISA and IMS; the polysaccharide-based adjuvant, Advax XLTM XL™ and the Poly(gamma-glutamic acid) adjuvant. Gerbu Pharma consists of biodegradable cationised lipid nanoparticles, whereas Montanide Pet Gel A (PGA) consists of a high molecular weight polyacrylic polymer in water. Finally, Sigma Aldrich Plc.'s Aluminium Hydroxide Gel was also included. Advax XL™ was developed by Dr. Nickolai Petrovski *et al* (Vaxine Pty Ltd., Australia) and formulated specially for the use in this trial. It consists of a nanocrystalline isoform of inulin, a polysaccharide consisting of a linear chain of fructose capped by glucose (Petrovski *et al.*, 2004). One viral antigen (killed African Horse Sickness Virus-AHSV) and one bacterial antigen (killed *B. mallei*) were used. Antigen-specific antibody responses and measures of reactogenicity (inflammation, skin thickness and pyrogenicity) were assessed.

A total of 18 camels were used, stabled at CVRL. Camels were checked that they had not been previously immunised with either *B. mallei* or AHSV prior to this study. Eight camels were injected with antigen-adjuvant mixtures on either side of the neck (AHS antigen on to one side, *B. mallei* on to the other side). Another 8 camels were injected with pure adjuvant (without antigen) on one side. The last 2 camels were injected with pure antigen (without adjuvant) on one side. Camels receiving adjuvanted-AHS4 vaccines were given a single booster dose three weeks post-inoculation, whereas camels receiving *B. mallei* vaccines were boosted every week up to a total of 5 booster doses post-inoculation. In a second trial, using *B. mallei* antigen, a six booster regime was applied. Adjuvants which caused severe inflammatory responses (Montanide ISA, Gerbu Vet and Gerbu Pharma) were left out since the six booster regime would be too traumatic to the camels. Only Montanide IMS, Montanide PGA, Alum, and Advax XL™ were used in this second trial.

Results

The oil-based emulsion adjuvants such as Gerbu Vet and Montanide ISA whilst enhancing antigen-specific antibody production, suffered from high levels of reactogenicity. By contrast, two newer particulate adjuvants, the polysaccharide-based adjuvant, Advax XLTM XL™, and the Poly(gamma-glutamic acid) adjuvant, Montanide PGA were not associated with significant reactogenicity. Of all the adjuvants tested Advax XLTM XL™, showed the most favourable overall response, enhancing high levels of specific antibody to both African Horse Sickness and *B. mallei* whereas Montanide PGA induced antibodies to only African Horse Sickness.

In general, it was observed that all adjuvants mixed with *B. mallei* produced more inflammation when compared to that of AHS. It can therefore be assumed that differences in severity are caused by the antigen itself. Antigens injected without adjuvants did not induce any inflammatory responses, or antibody production. This means that there was no immune response to antigens injected without adjuvants.

Conclusion

The main aim of this trial was to find the best adjuvant for antibody production in the dromedary camel. This adjuvant will have had to stimulate a high antibody titre to both a viral and bacterial antigen, without inducing a severe local reaction. The adjuvant Advax XL™ generated a high antibody titre to both bacterial and viral antigens and did not induce any significant inflammatory reaction. Montanide PGA and Alum both gave high antibody titres with the viral antigen without inducing inflammation. However, they did not stimulate an antibody response to the bacterial antigen. Montanide ISA, Gerbu Vet and Gerbu Pharma all produced a good antibody titre to the viral antigen, but generated severe inflammation at the same time. Montanide IMS, did not produce antibodies of any kind to both bacterial and viral antigens. Through this trial, Advax XL™ has therefore proved to be the best adjuvant because it produced antibody to both antigens tested, without inducing inflammation. Montanide PGA succeeded in this, with respect to viral antigen exposure, and therefore also has the potential to become a very useful adjuvant for the future.

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21. Identification of Nanobodies for Screening Breast Cancer Patients

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Introduction

Camelids have unique antibodies circulating in their blood (Hamers-Casterman *et al*., 1993). These so-called Heavy-chain antibodies lack an immunoglobulin light chain, which means that the antigen is recognized by one single domain, known as the nanobody. Using standard genetic engineering techniques we developed a strategy to clone a library of nanobodies from the lymphocytes of an immunized dromedary (or llama). By displaying the cloned nanobodies at the tip of bacteriophages, it becomes possible to select the antigen-specific nanobodies (Saerens *et al*., 2004). The selected, recombinant nanobodies have many useful properties for biotechnological applications and even for diagnostic or therapeutic applications. Because of their small size, the nanobodies are expected to diffuse rapidly throughout the body to reach their target organs, while the excess of nanobodies will be rapidly cleared from the body via the kidneys. Therefore radio-labeled nanobodies should be a potent tool to trace in vivo the presence of tumors in a non-invasive approach.

To test this idea, we decided to generate nanobodies against the human epidermal growth factor receptor or HER2 since human cancers of epithelial origin such as breast cancers are often overexpressing this membrane protein that is generally associated with poor prognosis. Potent therapeutic anti-HER2 monoclonal antibodies (Trastuzumab and Pertuzumab) are available, however, the use of these expensive therapeutics is only effective to treat the HER2 positive breast cancers. To determine the appropriate therapy, the HER2 status of breast tumors is currently assessed invasively in tumor biopsies. It is our objective to replace the invasive tumor biopsies by a non-invasive in vivo radioimmunodetection of HER2 positive tumors using nanobodies and single photon emission computed tomography (SPECT).

Materials and Methods

A dromedary was immunized with recombinant HER2 mixed in Gerbu adjuvant (Vaneycken *et al*., 2011). The generation of the library of nanobodies, the selection of HER2-specific nanobodies, the expression and purification of nanobodies and their biochemical characterization (yield, stability, affinity, epitope mapping) was as described by Saerens *et al*., (2005). Labeling of nanobodies with ^{99m}Tc was according to Vaneycken *et al*., (2011). The reactivity of the nanobodies to HER2 positive SKOV3 cells with either cold or ^{99m}Tc-labeled anti-HER2 Nanobodies, the biodistribution and tumor targeting potential was evaluated in nude mice bearing HER2 positive LS174T and HER2 negative MDAMB435D xenografts for two selected nanobodies (2Rs15d and 1R136d) via in vivo pinhole SPECT/micro-CT. The exact protocols are published in Vaneycken *et al*. (2011).

Results and Discussion

Forty nanobodies against HER2 were retrieved from the nanobody library cloned from an HER2-immunised dromedary. By biosensor measuremetns (Biacore T100) it was shown that these nanobodies have low nanomolar affinity for their cognate antigen. We then continued with those nanobodies that could be purified with a yield above 1 mg per liter of bacterial culture and lacking reactive amino groups (lysines) in their antigen binding loops. The nanobodies were ^{99m}Tc-labeled and purified to a final radiochemical purity of more than 99%. Saturation binding studies showed that ^{99m}Tc labeling was in general not associated with a great reduction of immunoreactivity. Most nanobodies (but not all) are apparently not competing for HER2 binding with the therapeutic antibodies Trastuzumab or Pertuzumab (Hoffman-La Roche).

The *in vivo* biodistribution of the labeled nanobodies indicated that tumor accumulation

varied between 0.78 and 4.44 percent injected radio-activity per gram of tissue (% IA/g). Three ^{99m}Tc-nanobodies tested in HER2 positive SKOV3 xenografts, have a tumor uptake of more than 4 % IA/g. Two of these show high tumor uptake (3.76±0.82 and 4.32±0.92 % IA/g) in HER2$^+$ LS174T xenografts, but low uptake in HER2$^-$ MDAMB435d xenografts (0.71±0.07 and 0.73±0.35 % IA/g). Importantly, apart from the specific tumor uptake and high non-specific renal uptake, all ^{99m}Tc-nanobodies displayed low non-specific accumulation in the liver, muscle and blood, resulting in high tumor-to-background ratios.

The potential of two nanobodies (2Rs15d and 1R136d) to recognize the HER2 antigen on LS174T cells (HER2 positive) or MDA-MB-435D (HER2 negative) cells when present in female athymic nude mice was tested by non-invasive in vivo imaging (Figure 1). These results demonstrate the potential of ^{99m}Tc-Nanobody 2Rs15d as tracers for non-invasive imaging of HER2 expressing tumors, and this nanobody is identified as our lead for a phase I clinical study.

Figure 1. Representative transverse, coronal and sagital views of fused SPECT/CT images of HER2$^-$ MDAMB435D and HER2$^+$ LS174T tumor-bearing mice 1h after i.v. injection of ^{99m}Tc-labeled nanobodies showing good tumor targeting in the HER2$^+$ tumorand poor tumor accumulation by anti-HER2 ^{99m}Tc-Nanobodies in HER2$^-$ tumor.
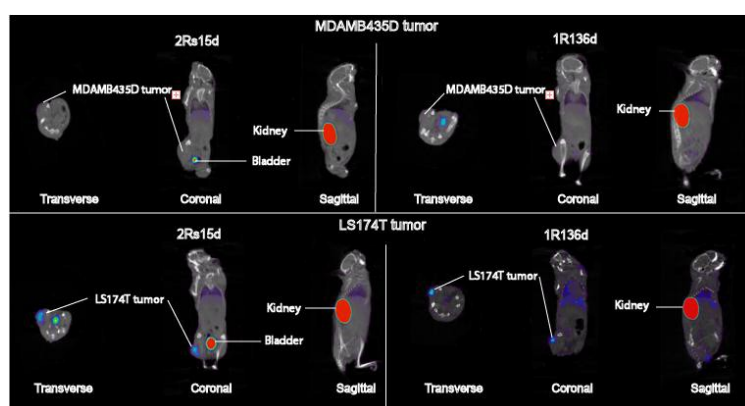
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Medicine
Infectious Disease
and
Health

22. Observations on Total and TCA-Soluble Plasma Copper Levels in Omani Camels During Winter and Summer Seasons

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Introduction

Seasonal variation in plasma Cu status has been reported in goats in Oman (Osman *et al*, 2003) and in Pakistan (Khan *et al*., 2007). Cu deficiency was reported in Oman in livestock (Ivan *et al*, 1990). This report aimed to study plasma Cu levels in Omani camels in winter and summer seasons.

Materials and Methods

Female camels, (n=25 in Winter and n=35 in Summer) all above 8 years of age, were picked at random from a large herd raised and kept for racing and festivals on a large farm north of Muscat. The herd was housed in half shaded good ventilated large pens. The camels were given in groups fresh alfa alfa (20 kg/head/d containing (mg/kg DM): 9.4 Cu and 566.3 Fe, and dates (5 kg/head/d containing Cu (7.57) and Fe (317.1) and mineralised salt blocks containing Cu (300) and Fe (1500), *ad libitum*. Fresh water was provided freely. The animals were dewormed twice a year. Total plasma and TCA-sol Cu in blood samples collected in heparinized vaccutaner tubes from the jugular veins of the camels, during winter and summer seasons, and in dried samples of dates and alfa alfa, were determined following methods described by Osman *et al*, 2003. Effects of season on measured TCu and TCA-sol (mg/l) and the calculated proportion of TCA-sol/TCu was studied using General Linear Model procedure using SPSS 19 (2010) personal computer package.

Results

In both groups of camels the mean TCu concentrations were within deficient to marginal levels (Table1). There was no significant (P>0.05) difference between means of TCu in camels bled in Winter and those bled in Summer (Table1). The mean of TCA-sol tended to be higher ($P = 0.075$) in Summer than that in Winter. The proportion of TCA-sol/TCu was higher ($P = 0.002$) in Summer than in Winter

Table 1 Means (± SE) of measured TCu, TCA-soluble (mg/l) and the calculated proportion of TCu/TCA-soluble Cu in camels that had their TCu within normal (> 0.699 mg/l) or low (< 0.70 mg/l) ranges, and the total group during winter and summer seasons

	Winter bleeding			Summer bleeding			Significance of season effect
	Total	Normal	Low)	Total	Normal	Low	
N	25	6	19	35	4	31	
TCu	0.55±0.05	0.76±0.06	0.47±.03	0.50±0.03	0.75±0.09	0.46±0.03	P<0.05
TCA-sol	0.39±0.04	0.60±0.07	0.30±0.04	0.46±0.03	0.72±0.08	0.42±0.03	*P*=0.075
TCA-sol/TCu	0.74±0.06	0.80±0.1	0.72±0.07	0.93±0.03	0.96±0.12	0.93±0.04	P=0.002

Discussion

The main feature observed in this study was that a large proportion of camels (76 and 89% in winter and summer, respectively), had their TCu within marginal to deficient levels, as suggested for cattle (Perry, T. W., 1980) in both seasons. Means of 0.9 – 1.0 mg/l were found in camels in Sudan (Naway, 1983), 0.86 ± 0.24 mg/l in Bactrian camels in China (Zong-Ping *et al*, 1994) and 0.9 – 1.0 mg/l for dromedary and 0.8 - 0.9 mg/l for Bactrian camels reported by the Zoological Society of London (Higgins and Kock, 1986). In closer regions serum Cu was reported in UAE as 61.1 µg/100 ml (Fey, *et al*., 2005) and 71.51±0.05µg/dL in Al-Shargia region in Oman (Eltahir, *et al*., 2010). The dietary Cu levels in the current study were adequate, which suggests secondary causes to affect these TCu levels in the studied region. Current dietary Fe level was considered high enough to interfere with Cu metabolism in cattle (Bremner, *et al*, 1983). Furthermore, the high TCA-sol/TCu, particularly in winter, suggests the presence of high Mo and S in these diets. The reduction in blood

Cu to the level of 0.28 ± 0.17 mg/l was also observed in Chinese Bactrian camels fed from pastures containing Mo at 4.8 ± 0.25 mg/kg DM and Cu at 6.5 mg/kg DM (Zong-Ping, *et al*., 1994). The results presented by Ivan *et al* (1990) in Oman provided a Mo level of 2.8 Mg/kg DM and 0.37 % Of S in alfa alfa samples. These levels were enough to affect the Cu metabolism in sheep (Suttle, 1974). The diets of fresh alfa alfa fodder further reduce the availability of dietary Cu in the presence of small increases in Mo compared to corresponding increases in hay (Suttle, 1978).

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23. Challenges of Veterinary Care in a Large-Scale Camel Dairy Farm and the Effect of Health Status on International Trade of Camel Milk (*Camelus dromedarius*)

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Introduction

Camels are well adapted to harsh conditions and can produce more milk than any other animal in the same environment. However, camel milk is produced exclusively in traditional farming systems with low productivity that can not guarantee constant milk for urban markets or for international trade. In addition, camels are mainly considered as packing or racing animals and their production potential is underestimated (Faye, 2005). There are only few examples where large numbers of camels are ―confined‖ to an intensive production system (Juhasz *et al*. 2009). Hence, knowledge on the effect of concentration and intensive management on the veterinary care, health status and fertility of large dromedary herds is limited. An integrated camel milk production, processing and distribution company was established in Dubai a few years ago that created the world's first ISO certified, large-scale camel milking farm. From a scientific point of view, the farm can be considered as an unique ―research‖ project on management, husbandry, veterinary care to evaluate the long term effect of intensification on a traditional species, the dromedary camel.In this presentation, we summarize the challenges facing our team to produce constant, good quality camel milk from healthy animals that is suitable not only for domestic consumption but also for international trade.

Materials and Methods

The farm had to overcome several challenges. The concentration of animals, establishment of production, development of farm facilities, increase in production and in number of camels have been going on simultaneously. While the health status of the production group was stable and secure, there has been continuous importation of new animals of unknown production and health history from various sources. This fact required the establishment of strict quarantine measure and the operation of 2 units (quarantine and production) under the same ―roof‖. Pre-purchase examination criteria were also established to maximize production potential and minimize the risk of introduction of new camels. The veterinary care has several aims/tasks in a large-scale farm such as (1) to control infectious and zoonotic diseases; (2) to prevent multi-factorial diseases; (3) to decrease losses caused by intensive management; (4) to recognize and treat sub-clinical and clinical conditions; (5) to increase production by reaching maximum ―production‖ life-span of healthy animals and (6) to maintain animal well-being (welfare) and harmonize it with the environment. In order to reach these aims several programs have been developed and the husbandry/management system is under continuous improvement. The Bio-security Program consists of (1) control of animal movement; (2) control of movement of people; (3) cleaning and sanitation; (4) pest control and (5) staff training, health care, hygiene. The Herd Health Management Program has 3 main elements such as (1) Disease Control and Health Program; (2) Animal Welfare and Well-being Program and (3) the Breeding and Reproductive Management Program. All these elements are documented in Standard Operation Procedures (SOPs) that are part of the Food Safety Management System (FSMS) Manual and are discussed in details in the presentation.

Results and Discussion

Since the opening of the farm in 2006, there was a gradual increase in the number of adult camels, followed by a sudden jump to 1500 animals in 2008. By now, the number of camels has been stabilized around 1300 animals. There were over 35000 different serological tests performed on samples from the farm. Some of these results have been published by Wernery *et al*. (2007). A total of 5.3 % (860/16240), 0.8 % (31/4093) and 0.4 % (9/2033) of the samples were positive serologically for Brucellosis, Tuberculosis and FMD, respectively. Camels positive for any OIE listed disease have been removed from the premises of the farm. We have been successful to keep the farm free of major infectious diseases. In 2010, the 466 clinical ceases in adult animals were distributed among the following disorders: mastitis (37.4%), abscess formation (20.6 %), metabolic & nutritional problems

(10.5 %), bacterial & viral infections (10.1%), injuries (7.9 %), reproductive & obstetrical problems (6.7 %), dermatitis (2.1 %) and others (1.5 %). Mortality has been 2.8 % per year (36 cases). Every year there are new diseases that may not reoccur in subsequent years. In 2010, 50 % of the losses (20 cases) were related to bacterial & viral infections. Most of these cases were caused by a newly emerged *Rhodococcus equi* infection (Kinne at al., 2011) coupled with chronic mastitis. The continuous control of ecto-parasites (Sarcoptic Mange, Ticks) and fungal skin infections (Ringworm) represent a constant challenge and takes a lot of effort.

Breeding management and high reproductive efficiency are vital on a large-scale farm in order to control seasonal changes and maintain constant milk production. During 4 breeding seasons, we achieved > 80 % end of season pregnancy rate. Live birth rate showed a decreasing tendency from 80 to 73 % from 2007 until 2011. In parallel, reproductive loss was increased during the 2009-2010 breeding season. Every year, increasing numbers of calves were born (165-498) that highlights the importance of neonatal management. Calf mortality has been below 10 % for several years. However, due to a new disease of unknown reason that caused CNS signs, calf mortality reached 25 % in the last calving season. Corrective measures have been taken to prevent the occurrence of the disease during the next season.

We conclude that it is possible to keep dromedaries in intensive management system without causing excessive stress to the animals, however, the incidence of multi-factorial disease is increasing. In general, the animal health status of the farm is good and supports the international trade of camel milk.

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24. Breed Variation in Serum and Tissue Copper, Zinc, Manganese and Magnesium of Camels (*Camelus dromedaries*) in Saudi Arabia

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Introduction

Minerals are very crucial for animal health and productivity by playing an important role in many physiological activities and their deficiency causes a variety of pathological problems and metabolic defects (Deen *et al*., 2004). The level of nutrition and mineral intakes is known to affect the production and reproducing ability of male and female camels (El-Bahrawy and El-Hassanein, 2011; Ali *et al*., 2010). A few scientific studies have shown some evidence of sensitivity of camels to trace minerals disorders as a result of deficiency or toxicity in the same way as other ruminants (Faye and Bengoumi, 1994). Faye *et al*. (1992), Faye and Bengoumi, (1994), Liu *et al*, (1994) reported several incidences of clinical mineral deficiencies in camels, which underestimated because signs of subclinical deficiencies may remain unclear for a long time. On the other hand, Wiener (1979) reviewed the genetic variation in the incidences of many mineral metabolic disorders regarding the deficiencies and imbalances. He concluded that animal breeds and strains differ in their mineral requirements with various concentrations in blood and tissues. Few studies conducted to evaluate the effect camel breeds on the minerals metabolism with no studies in Saudi Arabia.

This study was conducted to evaluate the levels of Zn, Cu, Mn and Mg in blood serum, liver, kidney and meat tissues. Samples were collected from two dominant breeds (Majaheem and Maghateer) raised under traditional semi intensive system in Saudi Arabia.

Material and Methods

Fifteen healthy male camels (*Camelus dromedaries*) from each breed, Majaheem and Maghateer, with an average age of 1.5+0.5 years old were used in this study. Before slaughtering, blood samples were collected from the jugular vein using vacutainer tubes without heparin. Serum was collected by centrifugation for 3000 rpm/ 15 minutes and prepared for mineral analysis by wet digestion. After slaughtering at Al-Riyadh abbatoir, liver, kidney and meat samples were collected using stainless steel surgical blades and prepared by ashing (550 C/ 5hrs) using muffle furnace and diluted by concentrated HCl and 0.1 M HCl in 25 ml volumetric flask. All prepared samples were analyzed for Cu, Zn, Mn and Mg by using Inductively Coupled Plasma Optical Emmission Spectrometer (ICP-OES).

Data were analyzed using SPSS as a complete randomized design and significantl levels were declared at P<0.05 or other wise noted.

Results and Discussion

There are no any reported studies comparing the differences between camels breeds in Saudi Arabia in term of their mineral metabolism and their ability to accumulate minerals in their tissues. Most of studies regarding camels focus on the levels of mineral in blood serum and the possibility of different mineral metabolism in camels. The results showed a significantly (P<0.05) higher Cu and Zn and lower Mn and Mg concentrations in blood serum of the Majaheem breed compared with the Maghateer. Copper and Mg concentration in Majaheem liver were significantly (P<0.01) higher, but had lower level of Zn when compared with the Maghateer breed. For kidney samples, a significantly higher Cu concentration was found (P<0.0001) and lower Mn (P<0.05) and Zn (P<0.05) were found for Majaheem compared with the Maghateer kidney samples. Furthermore, a significantly (P<0.05) higher Mg concentration and lower Cu (P<0.05) in meat samples from Majaheem breed when compared with the Maghateer breed meat samples. The inorganic matter percentages of liver, meat and kidney were significantly higher (P<0.05) for the Majaheem breed compared with the Maghateer (1.91, 1.2 and 1.37 vs 1.46, 0.76 and 1.07%, respectively). Most of the values regarding the concentrations of these minerals in the serum and tissues were within the normal levels according to the studies conducted by different researchers such as Mohamed (2004), Badiel *et al*. (2006), Bakhiet

et al. (2007), Busadah (2007), Kadim *et al*., (2008) and Eltahir *et al*. (2010). In conclusion, the results indicate a breed difference may exist for Cu, Zn, Mn and Mg metabolism as a heritable characteristic. Further studies are recommended in the area of genetic selection for tolerance of minerals disorders.

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25. Selenium Toxicity in the Dromedary Camels
Clinical Symptoms and Lesions

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Introduction

Selenium (Se) is a group VI element with chemical properties very similar to those of sulfur, it has been demonstrated as an essential element for ruminants. Selenium is required to prevent deficiency diseases such as white muscle disease, maintain growth rates of young animals and promote reproductive performance. Camel sensitivity to trace element imbalances has been reported (Faye and Bengoumi, 1994). Selenium is generally considered a highly toxic element and selenium toxicity may occur in camels through incorrect diet formulation or prolonged oral exposure to elevated dietary selenium (Se) in forage. The objectives of the current study were to determine effects of graded levels of soldium selenite intake on camel performance and to provide preliminary data on camel selenosis (clinical symptoms and lesions).

Materals and Methods

Twelve healthy young camels were obtained from local UAE breed, aged 2 years and were acclimated to experimental design for 15 days. During the acclimation period, camels were treated with a broad-spectrum antiparasitic compound. Animals were housed in groups of 4 and were fed with a similar basal diet composed of Rhodes grass (*Chloris Gayana*) with an average quantity of 3 kg DM and 2 kg of pelleted concentrate 10 % protein (Soya Bean Meal – Maize – Barley – Wheat bran – Molasses – Salt – Premix). Camels were provided water *ad libitum*. Oral individual doses of selenium : 8.16, and 16 mg per day were given as sodium selenite, corresponding respectively : 8 mg (i.e. 17.44 mg sodium selenite), 12mg (i.e. 26.16 mg sodium selenite) and 16 mg (i.e. 34.88mg sodium selenite). Selenium was given enrobed in dates every day at the same time for 90 days. Selenium supplementation was stopped at the time of apparition of chronic selenosisand camels returned to normal good health gradually. At day 45 one camel of each group was slaughtered and a second one at the end of the experiment (at day 90).

Urine and faecal samples were taken every month from each camel. A sample of 600 g was taken from each camel, dried for 48h at 65°C, grinded and stored in dark and cool place until selenium analysis. Total 24 hours urine of each camel was also taken, using a special plastic bag placed on the vulva, weighed and a sample of 20 ml was taken and stored at $–20^{o}$C up to selenium analysis. Selenium content of the camel basal diet and water was also assessed at the beginning, the middle and at the end of the trial. Nutriments were dried, ground and stored in a dark cool place until analysis. Hair was taken before slaughtering from the neck and other part of the camel were taken at day 45 and 90 using a stainless steel knife. Before organs sampling, the weight of each whole carcass and each organ were recorded. Samples from lung, heart, liver, spleen, kidney, pancreas, suprarenal gland, shoulder and femoral muscle, anterior limb bones, posterior limb bones, brain, intercostals muscles, diaphragm muscle and urinary bladder were collected. Samples from the tissues were fixed in 10% neutral buffered formalin for microscopic evaluation; others samples were stored at -80°C until selenium analysis.

Selenium was determined in organs, hair, face, urine, diet and water by Inductively Coupled argon Plasma – Atomic Emission Spectrometer (ICP-AES), Varian vista MPX-CCD simultaneous, using 11 points of standard curve of Accu Trace[TM] Reference Standard solutions from Accustandard[®] – USA. Quality Control Standard.

Results

Selenium analysis in water showed no selenium. Selenium content in diet was 0.49 mg/kg in concentrate, 0.15 in Rhodes grass. The daily feed intake was 2 kg of concentrate and 3 kg of grass on

average. Thus, the selenium intake provided by the diet was 1.43 mg per day for camels during all the experiment, the mineral mixture providing 8, 12 and 16 mg of selenium per day. According to the treatment, the total quantity of selenium provided in the diet was 9.4 mg/day for camels in group 1, 13.4 mg in group 2 and 17.4 mg in group 3. So, the dietary Se concentration varied between 1.7 (group 1) and 3.5 ppm (group 3) DM.

Selenium concentration in urine and feces varied between 33.2 and 2230.5 ng/ml with a mean value 646.6 ± 610.9 ng/ml and between 193.5 and 13487.4 ng/g DM with an average mean 2346.02± 2653.9 ng/g DM respectively. The urinary Se concentration was higher at month 3 in group 2 receiving 12 mg. Se concentration increased significantly starting from month 2 for 3 groups up to the end of the experiment for groups 1 (8 mg Se) and 2 (12 mg), but decreased at month 3 in group 3 (16 mg Se) when Se supplementation was stopped.

Clinical symptoms of selenosis appeared at week 2 (see annex1). Camels had visible reduced feed intake and weight loss, reluctance to move and tachypnea following minimal exercise. Alopecia was seen in 3 groups, but more extended in group 3 with rough skin. In groups 2 and 3, theurinary excretion increased and dark watery diarrhea was also observed. Tears with pale mucous were showed as well as an evidence of impairment vision. Fissured pads appeared in all groups but more pronounced in group 2 and 3. Consequently, camels in group 1 and 2 developed a vesicular stomatitis. Camels stood with their head down and neck extended, taking short, rapid, shallow breaths. The recovery period ranged from 1 to 2 weeks. Severity of clinical signs of disease and time to recovery varied and were dose dependent.

At necropsy all animals from the 3 groups showed gross lesions, characterized with severe pulmonary lesions with accumulations of serosanguinous fluid and foam in the trachea, bronchi, and bronchioles. The heart of these animals was soft and pale, all abdominal muscles, diaphragm and intercostals muscles were pale. The liver was red and mildly swollen. Heart, liver and kidney were congested and necroses. while pancreas was atrophied. Brain edema was observed in all treatment.

The major histopathologic changes camels that manifested clinical signs of selenosis included kidney lesions showed congestion in blood capillaries of cortex and medulla, degenerative changes in lining epithelial cells of convoluted tubules . Lesions were extended to other tissues with severe vacuolar degeneration in epithelial lining in urinary bladder and sub capsular focal hemorrhagic areas in spleen. Edematous fluid was seen in between the muscular fibers and slight congestion of blood capillaries in heart, hepatic cells, congestion in central hepatic vein and hepatic sinusoids. In addition, focal areas of muscular hyalinization (non –inflammatory) and edema were observed in intercostals and diaphragm muscles. Activation in lymphoid follicle was seen in cervical anterior lymph node. Focal hemorrhagic areas and blackish green fine granules accumulation were observed in focal areas of spleen. Brain showed perivascular oedema in brain.

Discussion

Se deficiencies have been reported in United Arab Emirates, camels are often supplemented with commercial Se and vit E compound; however, no data on camel selenosis have been reported. In this current study the amount of Se intake from basal diet is 1.43 mg Se per day i.e. 0.28 mg/kg DM that was considered approximatively the requirements for dairy cattle (NRC 2001). However, according to the mean weight of the camel in our study (183 kg), the selenium supply with the basal diet was 0.78 mg/100 kg LW. That was lower than recommendations for beef cattle (1 mg/100 kg LW). Selenium is needed in small amounts. The minimum level of selenium in diet that causes chronic selenosis in most animal species is 4-5 mg/kg DM (US NAS/ NRC, 1976) and the minimum level needed to prevent deficiency is 0.02 – 0.05 mg/kg DM (US NAS/ NRC, 1971). Excess Se intake can lead to Se poisoning, but species susceptibilityselenium toxicosis is variable. (Tiwary et al. (2006) did not observe lamb mortality with an oral sodium selenite up to 4 mg/kg LW. For other authors, the oral median lethal dose (LD50) of sodium selenite has been reported to be 1.9 ±1.2 mg of Se/kg LW (Blodgett & Bevill, 1987). A daily intake of 0.25 mg/kg LW was considered as toxic for sheep and cattle (Muth & Binns, 1964). These levels listed previously are higher than our dietary levels in the present study (0.051 to 0.095 mg/kg LW), which seems to show a high sensitivity of camel species to Se toxicosis. A limit marrow is to be considered between selenium requirement and toxicity. In this study, lesions appeared with a selenium intake of approximatively 2.5 mg/kg DM, while typical lesions of chronic selenium toxicosis were observed on young cattle receiving more than 5 mg/kg DM for 120 days (O'Toole & Raisbeck, 1995). The clinical symptoms showed in this study were in accordance with previous signs observed in chronic poisoning in other species (Casteel et al., 1985;

Tiwary *et al.,* 2006). The necrosis of camel pad was comparable to those occured in chronic selenosis in cattle (O'Toole & Raisbeck, 1995) and horse (Raisbeck *et al*., 1993).

Conclusion

The results of this study indicate that the camel is sensitive to excess Se intake and selenosis, occurs with high-level selenium intake. Young camels are very sensitive. Clinical toxicity symptoms were observed at a dose of 8 mg Se daily within 3 weeks under sodium selenite form. According to dietary Se supply and to mean weight of the animal from the group 1, selenosis appeared with 0.05 mg/kg LW Se supply only. Severe intoxication occurred with 16 mg Se supplementation, i.e, 0.10 mg/kg LW. Those values were 5 times less than for sheep and cattle. According to such results, it could be important to limit Se supplementation in camel at 0.01-0.02 mg/kg LW, i.e. approximatively 4-8 mg per day for adult animals or 0.5-1 ppm in the diet.

Although meeting dietary selenium requirements is an important nutritional requirement for camels, mineral supplementation may also enhance the nutritional quality of the camel product (milk and meat).

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Annexes 1 - Clinical symptoms

Alopecia   Pad leions   Sternal   Diarrhea   Hypertrophy of
                                          Position   Cervial lymphnoid

Necropsy findings

Heart   Liver   Pulmunary   Brain Edema   Spleen   Kidney   Muscle
Congestion,   Congestion   Congestion   Congestion   Necrosis   discoloration
Necrosis,
Soft Discoloration

Histopathology findings
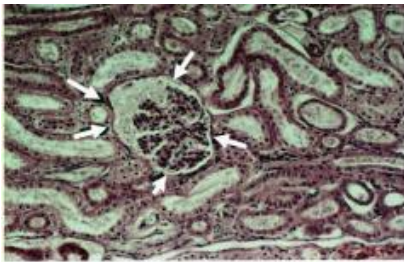
Kidney: degenerative changes in
epithelial lining cells
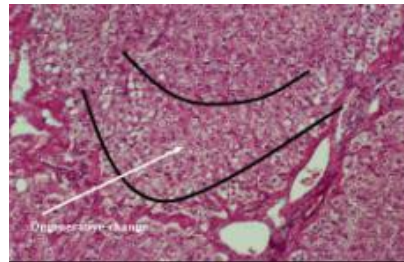
Liver: sinusoids congestion, degenerative changes
in periportal zone hepatic cells
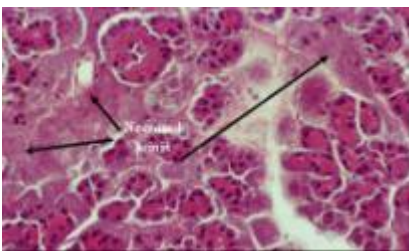
Pancreas: necrotic areas and fibrosis Heart: degenerative changes in myofibers
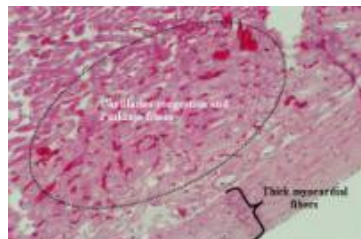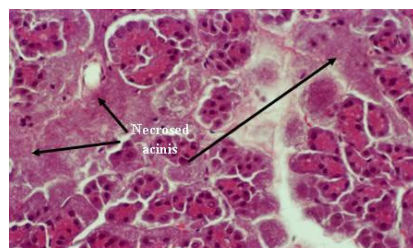
26. Sero-Epidemiology and Mapping of Johne's Disease (Paratuberculosis) in Camels (*Camelus dromedarius*) of the Sultanate of Oman

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Introduction

Johne's disease (JD) is a chronic wasting enteritis of domestic and wild ruminant species (Chacon *et al*., 2004) characterized by unresponsive diarrhea, loss of condition and emaciation. The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and is endemic worldwide in domestic livestock with scanty reports in camels (Mustafa, 1987; Alluwaimi, 2008 and Ameen and Ahmed, 2010). Transmission of the disease is through contact and usually infection is acquired during calfhood but prolonged incubation time prevents the appearance of clinical disease before 1-2 years of age (Collins, 2003).

Paratuberculosis is a disease of global economic importance for meat and dairy economists and its control presents a global challenge to livestock industry (Ott *et al*., 1999). Moreover, some studies indicate a possible link between MAP and human Crohn's disease that highlight a potential public health hazard (Bull *et al*., 2003). Control of JD is based upon successful implementation of test and slaughter policy, improvements in calf rearing and hygiene of herds and vaccination (Daniels *et al*., 2002). Serological diagnosis is the mainstay of JD control programs and recent studies indicate the potential of using commercial enzyme linked immunosorbent assay (ELISA) in camels (Allawami 2008; Ameen and Ahmed, 2010). The present study was designed to map the prevalence of Johne's disease in the camel population of Oman.

Materials and Methods

A cross-sectional serological study was planned to investigate the prevalence of JD in the camels of Oman. Sample size calculation was performed at expected disease prevalence to be 50%, 95% confidence interval and 5% error margin (Thrusfield, 2005). Serum samples from 2255 (254 males & 2001 females) randomly selected camels from 525 geographically marked holdings were collected. Georeferenced information was recorded on ArcPad™ (ESRI, USA) mounted Juno™ SB handheld computers (Trimble Navigation Limited, USA) and a geodatabase was built. The samples were further categorized in 4 age groups (1-4) viz. less than equal to 2 years (n=402), 2.1-5 years (n=509), 5.1-10 years (n=914) and more than 10 years (n=430) of age to investigate the age related dynamics of the disease. Samples were subjected to a commercial ELISA kit (LSIVET Ruminant Serum Paratuberculosis Advanced, France) as described by Ameen and Ahmed (2010). Data was analyzed by using IBM SPSS Statistics 17.0 for Windows® (IBM Corporation, New York, USA).

Results and Discussion

Differences were observed in the prevalence of Johne's disease among camel herds of sampled areas, $\chi2=16.6$, 5df, p<0.01 (Map-1), with highest value observed in Al Buraimi (18.6%) followed by Dhofar (13.5%), Al Wusta (10.3%), A'Dhahira (5.0%), Dakhiliyah (2.4%) and Ash Sharqiyah (1.1%). The prevalence rate varied from 50.0 to 0.7% in positive holdings and 36.8 to 3.2% in various wilayats of Oman. Overall prevalence of Johne's disease in camels in individual location was found to be 2.6% (n=59) which was highest in the camels of Al Buraimi (5.7%) followed by Al Wusta (3.7%), Dhofar (2.9%), A'Dhahira (1.5%), Ash Sharqiyah (0.8%) and Dakhiliyah (0.1%), $\chi2$ (5df)=14.27, p=0.01. The prevalence was higher in areas where owners adopted more conducive practices for the intra-animal transmission (overcrowding, lack of hygiene, placement of feeding and watering utensils at ground level) of MAP (Daniels *et al*., 2002). Higher prevalence was observed in female (2.8%) camels as compared to males (0.8%). However, this prevalence was not affected by the sex of animals (p<0.05) and similar results were reported by Pence *et al*. (2003). Prevalence of MAP was significantly different ($\chi2=8.70$, 3df, p=0.03) in sampled age groups with highest prevalence was found in group-4 (4.4%) followed by group-3 (2.5%). 2 (2.4%) and 1 (1.2%). Although the newborn

animals and those in their first 6 months of their life are most susceptible to acquire JD, usually sero-conversion and clinical disease appears in later years of life (Collins, 2003).

The study mapped the country wide seroprevalence of Johne's disease in camels of Oman which is comparable to a study in camels of neighboring Saudi Arabia (Ameen and Ahmed, 2010) and indicated a need to study detailed epidemiology of the disease that will help in devising a national control program.

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Map-1 Herd based prevalence of Johne's disease in camels of different governorates and regions of Oman
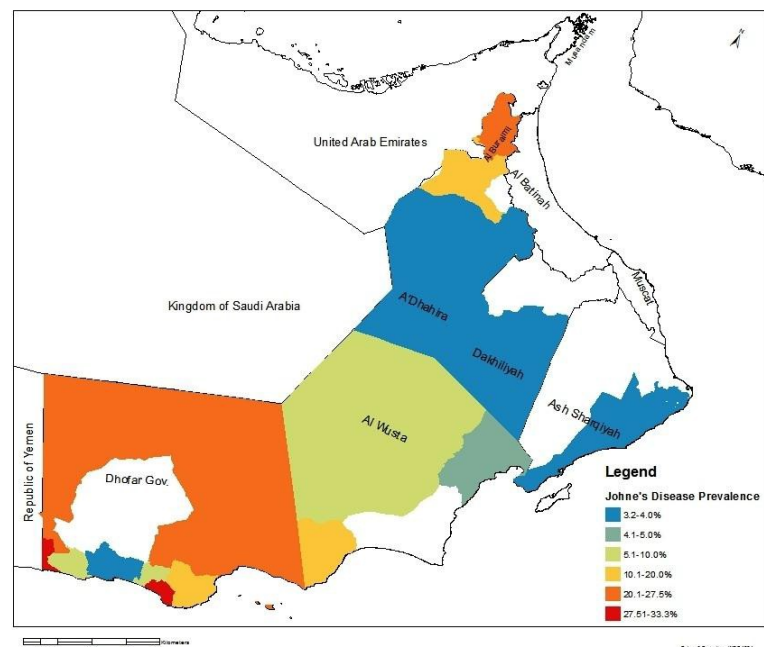
27. Sero-Epidemiology and Mapping of Brucellosis in Camels (*Camelus dromedarius)* of the Sultanate of Oman

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Introduction

Livestock brucellosis causes direct and indirect losses through abortions, still birth, metritis and up to 25% of milk reduction. It is an important re-emerging communicable disease in the Middle East and Mediterranean countries (Saleem *et al*., 2010). Rose Bengal plate agglutination test (RBT) and Enzyme-Linked Immunosorbent Assay (ELISA) are among some widely used serological assays to detect antibodies against Brucellosis (Oktay *et al*., 2011). Currently, indirect and competitive ELISA formats are preferred methods for screening and surveillance purpose (Perret *et al*., 2010). The disease has been reported frequently in camels where they were in contact with infected small ruminants (Erdenebaatar *et al*., 2004; Dawood, 2008). The incidence of clinical disease is very rare in camels but the possible shedding of organism in milk may lead to the transmission to humans (Corbel and WHO, 2006).

Brucellosis caused by *Brucella melitensis* is endemic in Oman and the records from the Veterinary Research Center (VRC) and Ministry of Health (MOH) indicate endemic livestock and human brucellosis in the Southern Dhofar governorate (Al Ismaily *et al*., 1989, Anonymous, 2010). However, the prevalence of brucellosis in the camel population of the Sultanate was not established and the current study was planned to map this prevalence.

Materials and Methods

For the current study, 525 randomly selected geographically marked animal holdings were randomly selected and 2255 (254 male & 2001 female) camels from these locations were bled for the collection of serum. Sample size was calculated at the expected disease prevalence of 50% (unknown), 95% confidence level and 5% error margin (Thrusfield, 2005). Georeferenced animal credentials were collected on ArcPad™ (ESRI, USA) mounted Juno™ SB handheld computers (Trimble Navigation Limited, USA) to build a geodatabase. Samples were further divided in 4 age groups (1-4) viz. $\leq$2 years (n=402), 2.1-5 years (n=509), 5.1-10 years (n=914) and >10 years (n=430) of age. Initial screening was performed through a commercial Rose Bengal plate agglutination test (RBT, Anigen, Animal Genetics, Inc) and positive samples were then subjected to a commercial competitive ELISA (Compelisa, VLA, UK) for confirmation (Perret *et al*., 2010). Data was analyzed by using IBM SPSS Statistics 17.0 for Windows® (IBM Corporation, New York, USA).

Results and Discussion

Overall herd-based seroprevalence was recorded as 1.4% (n=8) with highest prevalence found in camel holdings of brucellosis endemic in Dhofar governorate (3.7%, n=6) followed by Al Batinah (2.5%, n=1) and Ash Sharqiyah (1.3%, n=1) regions, χ^2=2.05, 2df, p=0.36 (Map-1). Endemic nature of brucellosis (Al Ismaily *et al*., 1989), more inter-species contact and pasture sharing could be the cause of higher prevalence in Dhofar (Corbel and WHO, 2006).

Individual prevalence of 0.4% (n=9) was recorded in the camels of Sultanate and difference observed in this prevalence between camels of Dhofar (0.8%, n=7), Al Batinah (0.8%, n=1) and Ash Sharqiyah (0.4%, n=1) was not significant (χ^2=0.44, 2df, p=0.80). All the seropositive camels were of female sex (0.5%) and belonged to local breed. The prevalence was not affected by the age of camels (χ^2=0.44, 2df, p=0.80) and marginally higher prevalence (0.5%) was recorded in camels belonging to age group-3 & 4 as compared to group-2 (0.4%). Within-herd prevalence varied from 20-1.7% in camel herds of positive areas. Majority (75%, n=6) of the positive camels were kept with small ruminants that favored the inter-species transmission of disease (Erdenebaatar *et al*., 2004; Dawood, 2008).

Seroprevalence of brucellosis in camels was mapped for the first time in Oman during the study and suggested that camels should be included in the brucellosis surveillance and control program in the Sultanate.

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Map-1 Seroprevalence of brucellosis in camel herds of various governorates and regions of the Sultanate of Oman.
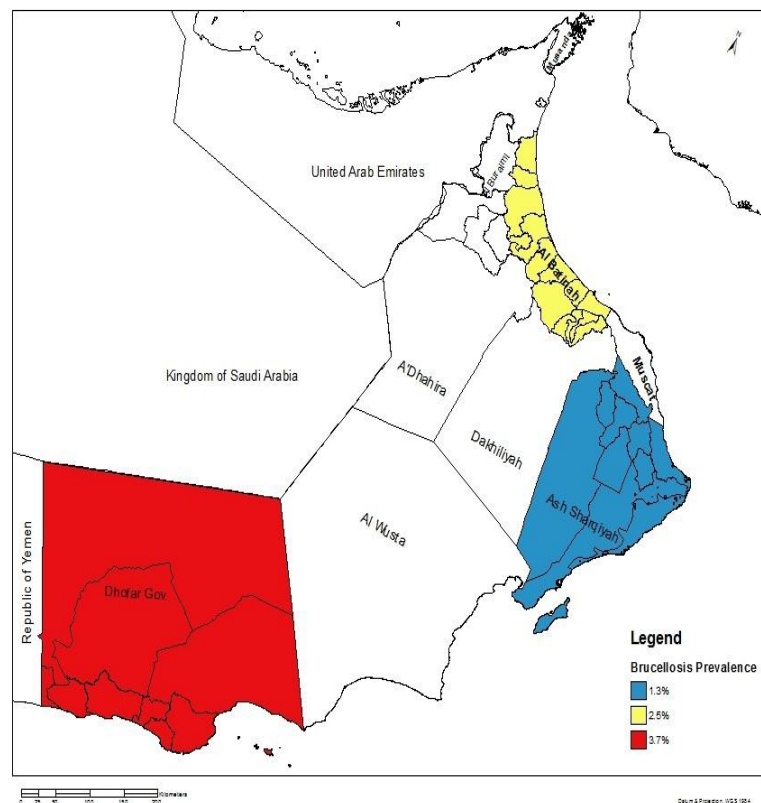
28. *Wasm* - An Ethnoveterinary Practice for Treatment of Camels in Oman

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Introduction

Camels are an important animal species that provide milk, meat and transportation for humans, and in recent times are used as sport animals. Ethnoveterinary medicine deals with folk beliefs, knowledge, skills, methods and practices pertaining to the health care and welfare of animals (Mathias-Mundy and McCorkle, 1989:3,Quiroz, Consuelo 1996). *Wasm* with a red-hot iron, stone, or potsherd is (McCorkle, C.M and Martin, M. 1998) an ancient practice and is utilized by experienced healers to treat a variety of camel ailments (Abbas *et al* 2002). As a healing art, it appears to be a routine and multi-purpose technique among all West African pastoralists (McCorkle, C.M. 1986). Cautery as a treatment modality for several human ailments is popular among Arabs. In Oman, it has been used for hundreds of years to treat a broad spectrum of human disorders. It is mentioned in the *hadith*, in a saying by the Prophet Mohammed (PBUH) stating that cure lies in three: a mouthful of honey, scarification by a cupping expert and cautery by fire (Ghazanfar, 1995). In many ancient civilizations, hot-iron branding was employed as a means of individual animal identification (Bowling *et al.,* 2008). The present study was undertaken to initiate the gathering of a body of information relevant to ethnoveterinary practices in Oman.

Materials and Methods

Information was collected from three traditional healers who have practiced hot iron *wasm* on camels to treat a number of musculo-skeletal disorders. Their experience with *wasm* varied from more than two decade to nearly five decades. The information was collected using a well-structured questionnaire, complemented through open-ended interviews that lasted approximately one to two hours. The three healers were from the Batinah region.

Results

The practice of utilizing *wasm* on camels is based on the religious belief of the healers and the traditional knowledge that they acquired from their fathers/ and forefathers. They treated on an average 10-15 cases a month, most of which occurred during the winter months when camel races were frequently held. A partial list of the types of conditions treated is shown in Table 1. They evaluated the animal's condition by conducting visual assessment of the camel, while it was sitting, standing, walking, and during a slow run. *Wasm* was applied to the camels while being restrained in the standing, sternal or lateral recumbency positions. Camels of all ages, including, pregnant she camels were treated. They utilized thin metal rods that were pointed to varying degrees or blunt ended. The healers used a marker pen to draw actual sign on the skin prior to the *wasm* application. The coal burner was heated sufficiently to assure that the metal rods were red hot. Different signs and shapes were utilized for *wasm* based on the healer's personal judgment of the severity of the inflammatory condition. Most often four signs were employed, such as a straight line of varying lengths (-), period (.), a plus sign (+), and a cross sign (X) (Table 1).Thereafter topical dressing ointments and sprays were applied to protect the wound and stall rest was advised for 3- 10 days. The healers were in agreement that the success of the treatment depended upon the accuracy of the initial diagnosis and the time point of intervention that *wasm* was employed during the inflammatory process. One healer performed hot iron cauterization occasionally on humans. They possessed knowledge on a wide range of wasm signs that are typical for the tribes of Oman that applied them on camels as a mark of ownership and identification.

Discussion

The traditional healers treated a variety of inflammatory musculo-skeletal disorders such as muscle sprain, strains on joints, effusion/edema of joint spaces and tendoarthritis. The success or recovery through this treatment is dependent upon localized immuno- and haemato-genic responses (McCorkle, C.M and Martin, M. 1998). Similarly, western African pastoralists treat livestock sprains

with a series of tiny burns in the sprained area much like the "pinfiring" performed on Western racehorses with leg problems, to increase blood flow to the injured part (McCorkle, C. M. (1986). Cauterization has also been practiced in Germany and its applications have been described by Berge and Westhues (1961) to treat disorders such as chronic tendinitis and tendovaginitis, various forms of chronic arthritis and periostitis. According to these authors, the treatment results in a local acceleration of the reabsorption of tissues that have undergone chronic inflammatory alterations. Cauterization works as a counter irritant that might stimulate movement of joints in chronic tendoarthritis (Abbas *et al* 2002). The traditional healers did not attempt to treat all medical conditions of camels with *wasm*. Trypanosomiasis and mange for example were treated with orthodox veterinary medicines.

Table 1: Medical conditions treated with *wasm* and the ways (signs) in which *wasm* is applied

Medical conditions	Signs of *wasm*
Hatham – Wry neck syndrome, with severe muscle spasm and occurs high in winter season	*wasm* as | or as |.|. serially on each cervical vertebrae on both sides of the neck.
Khahal- Sprain on the thoracic group of muscles.	*wasm* as | on chest of varied lengths 5-7.5 cm on each side.
Fiijar- Post race/exercise colic signs, off feed and conditions associated with lengthy fecal pellets.	*wasm* as | just next to the umbilicus of varied lengths either one as on ventral midline or two || each on the para median line on both sides.
Lian- Fetlock knuckling of forelimbs-Toe pads not kept straight- which occurs in camel calves, ages varied from 4 months to two years old. They believe this condition is related with copper and sometimes calcium deficiency. Prior to *wasm* they treat with copper boluses (Cupric oxide needle capsules) & if not recovered, they proceed in applying wasm.	*wasm* as (**..**) period sign on the anterior side and (**..**) two on posterior border of the fetlock.(two healers) Or *wasm* as | | only on the posterior border of the fetlock. (one healer)
Argaht Thifnai- Lameness and non -weight bearing of the affected hind limb either at the level of stifle or at hock. *Shanoot* – Lameness of hind limbs and strain on stifle/ with observance of intermittent lameness associated with a change of pace while walking.	*wasm* as X on the medial side of stifle or at hock. *wasm* as ╪ on the mid lateral thigh and may extend up to stifle pad.(two healers) or *wasm* as (on just above the stifle pad of the affected limb. (one healer)
Mesah- Lameness due to inflammation of shin which may be equated to sore shin/inflammation around large metacarpal area. *Bursome* - Swelling and intense pain on the first phalanx (pastern)/ or just behind the 2nd and 3rd phalanges (just posterior to the toenails)	*wasm* as | on the lateral border of the shin of the affected forelimb. *wasm* as | applied just below the ears to the contralateral side of the affected limb.

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29. Occurrence of Cystic Hydatidosis in Camels *(Camelus Dromedarius)* in Dhofar, South Region of Oman

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Introduction

Hydatid disease or cystic echinococcosis caused by infection with the larval (metacestode) stage of *Echinococcus granulosus*, (*E. granulosus*) is considered to be one of the most important helminth zoonoses (Ibrahim *et al.* 2002). This disease has a worldwide importance and is widespread in different countries of the world including Middle East (Sadjjadi, 2006). Hydatid disease is endemic in most gulf countries: the incidence rate (3.6/100,000) was studied in Kuwait by Alkarmi in 1997. In Oman, a few seropositive cases of hydatidosis have been reported from the Dhofar region in humans and in camels (Idris *et al*. 1999; Sadjjadi 2006). In addition, many studies in Saudi Arabia were conducte in slaughterhouses targeting different livestock species, such as camels, sheep, goats and cattle (El-Metenawy, 1999; Ibrahim, 2010). *E. granulosus* has the ability to adapt to different host species that contributes to the broad distribution of this parasite; and probably due to this wide spectrum of hosts there is a great genetic variability among *E. granulosus* strains (Thompson and McManus 2002). In addition, humans can be infected by ingesting parasite eggs from the faeces of definitive hosts like dogs, foxes and other carnivores harboring the adult worms of echinococcus in their small intestine (Siles-Lucas and Gottstein, 2001). This study was aimed to determine the prevalence of cystic hydatidosis infection in slaughtered camels at Dhofar region, effect of age and sex on it, most common sites of infection and the percentage of hydatid cyst fertility. The study also utilized the samples to standardize the PCR as a molecular tool for the detection of *E. granulosus* at the Veterinary Research Center (VRC).

Materials and Methods

Suspected organs (liver, lungs) for the hydatid cysts were obtained from camels slaughtered at Salalah Municipal Center Slaughterhouse. The specimens were recorded in a special performa, preserved in a cool box and sent to the VRC for isolation, morphological examination and molecular detection of *E. granulosus* by using PCR. The infected organ samples were observed and the fluid was aspirated by a sterile syringe in sterile tubes. The hydatid fluid which contains protoscoleces was collected in sterile tubes and centrifuged for 15-20 minutes, washed three times with PBS, added with 95% ethanol (v/v) and preserved in -70C for molecular analysis. In addition, to understand the age related dynamics of CE in infected camels, animals were categorized in 3 groups viz., group-I (less than 3 years of age), group-II (3 to 5 years of age) and group-III (more than 5 years of age).
Polymerase chain reaction (PCR) was standardized for the detection of *E. granulosus* using specific primers and standard kit following manufacturers' protocol. The protoscoleces obtained from cystic fluid were used for DNA extraction using GenScript tissue direct multiplex PCR kit following manufacturer's instructions. The supernatant obtained from the protoscoleces was used for PCR-based detection of *E. granulosus* using mitochondrial 12S rRNA gene, with the primers sequence as follow:

For *E. granulosis*
Eg1f (5/_-CATTAATGTATTTTGTAAAGTTG-3/_)
Eg1r (5/_-CACATCATCTTACAATAACACC-3/_)
For the negative control *E. multilocularis* primers were used
EM-H15 (5/_-CCATATTACAACAATATTCCTATC-3/_) EM-
H17 (5/_-GTGAGTGATTCTTGTTAGGGGAAG-3/_)
PCR products were electrophoreses at 80 V in 1.2 % agarose gel for approximately 60 minutes using 1X Tris Boric acid (TBE) buffer containing ethidium bromide (0.5μg/ml) along with a DNA molecular size marker.
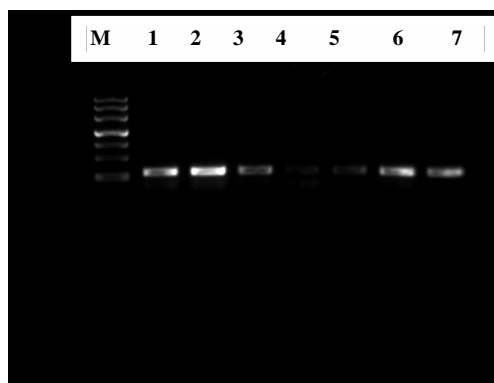
Fig 1. Postive PCR product (255 bp) for *E. granulosus* in lanes 1, 2, 3,4,5,6 and 7. Lane M is marker

Results and Discussion

Although, the percentages of positivity in male (52.3% n=34) was higher than female 47.7% (n=31) but no significant differences (P<0.05) was observed. This result has indicated that there is no effect of the sex on hydatidosis in camels. This finding was in agreement with the study of Fathi *et al.* (2011). However, the distribution of the hydatid cyst between organs was found to be significantly different (P<0.05). The lungs were found to be more infected (70.8% n=46) as compared to the liver (7.7% n=5) and this is in agreement with the previous findings of Ibrahem and Craig (1998). In addition, lungs and liver possess the first great capillaries sites encountered by the migrating echinococcus oncosphere (hexacanth embryo) which adopt the portal vein route and primarily negotiate hepatic and pulmonary filtering system sequentially before any other peripheral organ is involved (Ibrahem 2010; Kebede *et al.*, 2009). The majority of isolated cysts were found to be fertile (81.5% n=53) especially from lungs, whereas 13.8% (n=9) were recorded as sterile (without protoscoleces) and 4.6% (n=3) were calcified. Age related dynamics of CE in infected camels has indicated that, samples from older camels (age group-III) were more infected (63.1%, n=41) with hydatid cysts followed by the age group-II (21.5%, n=14) and age group-I (15.4%, n=10). This could be due to the reason that adult animals are exposed longer to the eggs of *E. granulosus* than young ones (Himonas 1987).

The present study provides a clear evidence for the presence of cystic hydatidosis in camels in southern region of the Sultanate. However, the investigation was conducted during five months period only (August 2010 to December 2010). Further studies are required to view the complete picture of the situation and the distribution of cystic hydatidosis in other regions of the Sultanate. In addition, the occurrence of cystic hydatidosis in other intermediate (human, cattle, sheep and goats) and final hosts (dogs and cats) should be taken into consideration before devising any control measures.

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30. Sero-Prevalence of Cystic Echinococcosis in Camels (*Camelus dromedarius)* in the Sultanate of Oman: A Preliminary Investigation

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Introduction

CE is a zoonotic infection caused by adult or larval (metacestode) stages of cestodes belonging to the genus Echinococcus and the family Taeniidae. Four species of *Echinococcus* are recognized, namely *E. granulosus*, *E. multilocularis, E. oligarthrus* and *E. vogeli* (WHO/OIE, 2001) and a new species, namely *E. shiquicus* was discovered from China by Xiao *et al*. (2006). This disease appears to be endemic and widely distributed in most of the Mediterranean countries such as Morocco, Tunisia, Libya, Israel, Syria, Jordan and Turkey, but the complete and accurate picture of its prevalence in human and animals is rather difficult for many reasons (Craig and Pawlowski, 2002). Although global epidemiological information on CE is not available, at least 100 countries have reported cases (Eckert and Deplazes, 2004). In rural areas, transmission of CE in humans and animals usually occurs where offals from slaughterhouses, farms or households are incorrectly disposed (Ibrahem, *et al*. 2002). Serological tests for CE in farm animals are of limited use and not available commercially due to cross-reaction between echinococcus and Taenia species. However, EgAgB has been assessed in ELISA and appears to have potential for improved immunodiagnostic of CE in humans (Wen *et al*. 1994; Ito *et al*. 1999). Crude hydatid fluid from camels is an important source of *E. granulosus* antigens, and has been used in affected countries such as North Africa and in Middle East where there is a high camel population and CE is highly prevalent (Ibrahem, *et al*. 2002). Furthermore, Antigen B (EgAgB) is a major protein produced by the metacestode cyst of *E. granulosus* and plays an important role in modulating host immune responses (Zhang, *et al*. 2010). This protein is highly immunogenic and can be detected by more than 80% of sera from patients infected with CE (Zhang, *et al*. 2003). Serological studies on CE in camels using EgAgB were undertaken in Libya by Ibrahem, *et al*. (2002) and in Saudi Arabia by Haroun, *et al*. (2006). In Oman, no studies have been carried out to determine the sero-prevalence of CE in domestic intermediate hosts. Hence, it becomes increasingly important to assess this situation and determine the true prevalence of CE. This study was aimed to develop an EgAgB-ELISA from crude hydatid cysts to determine the sero-prevalence of CE in camels from all regions of the Sultanate.

Material and Methods

A total of 706 serum samples from camels were randomly collected from camels in each region (Al-Batinah, n=35; Dakhiliyah, n=40; Ash Sharqiyah, n=74; Al-Wusta, n=136; A'Dhahira, n=51; Al-Buraimi, n=40; Dhofar, n=328 and Muscat, n=2). The studied regions are differing in their climate, geography, livestock population, husbandry and human activities. The positive and negative control sera were obtained from camels infected with CE from Salalah (Dhofar) slaughterhouse.

E. granulosus AgB enriched fraction was prepared from hydatid cystic fluid (HCF) obtained from naturally infected camels from slaughterhouse and the sera were tested according to a method described by Ibrahim *et al*. (2002).

Results and Discussion

Out of 706 camel sera, 158(22.4%) were sero positive for the antibodies of *E. granulosis*. The highest percentage of seropositivity (130/328, 39.6%) was recorded from Dhofar followed by Al-Wusta (16/136, 11.8%), Al-Buraimi (4/40, 10%), A'Dhahira (4/51, 7.8%), Ash Sharqiyah (3/74, 4.1%) and Al-Batinah (1/35, 2.9%). No cases sera were recorded from Muscat and Dakhiliyah regions. These results suggest that, CE is endemic in most regions of the Sultanate especially in Dhofar. This can be explained by environmental conditions that are conducive to the perpetuation of the parasite in this region which are absent in the other regions of the Sultanate. In addition, Dhofar is the only region in Oman that has a substantial amount of rainfall from the southern monsoon

‗Khareef' and has lower environmental temperatures compared to the other regions of the Sultanate. The average annual rainfall is about 110 mm but can range from about 70 to 360 mm. from July-August. In a study conducted by Wachira *et al*. (1991) to determine the transmission dynamics of CE, they found that eggs of *E. granulosus* could survive only a few hours under the high ambient temperatures of Turkana. However, when these eggs are exposed to sunlight and high temperatures, they became desiccated and did not hatch even when consumed by intermediate hosts. On the other hand, another explanation for the possible higher exposure of CE in this region might be attributed to the fact that most of animals are slaughtered at farms, houses or rural abattoirs in traditional conditions for family and religious occasions. At these places hygienic conditions are not met that leads to improper disposal of organs as explained by Azlaf and Dakkak (2006). Furthermore, the stray dog population and the close contacts with animals may also assist the transmission of CE in the affected region especially in rural areas. In the case of Al-Buraimi and Al-Wusta, the percentage of seropositive animals was closely similar and could be attributed to stray dog population in these areas and poor slaughtering management. These findings are fundamental for determining the prevalence of CE in camels of Oman and further study based upon molecular characterization of *E. granulosus* in other intermediate hosts (domestic animals and human) is warranted for planning control measures based upon stray dog population control, improvements in abattoir hygiene and awareness campaigns.

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31. The First Cases of Lancet Fluke *(Dicrocoelium Dendriticum)* Infections in Alpacas in Sweden

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Introduction

Dicrocoelios also known as the `small liver fluke disease`, is caused by the lancet liver fluke (*Dicrocoelium dendriticum*), which has a complex lifecycle that comprises two intermediate hosts, terrestrial snails of the genera *Cochlia* and *Zebria* and ants belonging to the genera *Formica* and *Lasius (*Gunsser *et al*., 1999, Bornstein, 2002*)*. Both intermediate hosts thrive on dry lowland regions as well as on certain dry mountainous areas with dry, chalky and alkaline soils.

The main end hosts are domestic and wild ruminants worldwide. It can infect other hosts e.g. hares and even humans (Bengtsson *et al*., 1968, Otranto and Traversa, 2002). Commonly, the infection runs a subclinical course in ruminants although liver lesions may be so significant that affected livers in sheep and cattle are condemned at slaughter. Occasionally, dicrocoeliosis can be fatal due to the impairment of the infected liver.

Dicrocoelios has been reported in alpacas and llamas in Switzerland and Germany (Gunsser *et al*., 1999, Otranto and Traversa, 2002, Wenker *et al*., 2001). However, dicrocoeliosis has not been reported in alpacas in the South American Andes as the intermediate hosts are not found in the Andes (pers comm. A Chávez de Garcia).

Materials and Methods

On an early November morning in 2010 a 2.5 year old pregnant alpaca was found dead on the pasture which was grazed by altogether 10 alpacas (herd A) since one year. Previously, the pasture had been grazed by cattle. At post mortem extensive liver lesions were seen and the lancet fluke (*D dendriticum*) were found.

Individual feacal samples of the 9 remaining animals in the herd (A) were analysed at the National Veterinary Institute (SVA) as well as feacal samples from two other alpaca herds (B & C) whose animals originated from herd A (by sedimenation according to Telemann). Herd B consists of three male alpacas and herd C of six alpacas born between 2001 and 2010.

Results

At postmortem it was seen that the alpaca had been of fair body condition and in early pregnancy at the time of death. The amount of digesta in the intestines was smaller than normal. About 75% of the liver parenchyma was affected by a chronic ongoing infection; chronic cholangiohepatitis, extensive biliary fibrosis, with interspersion of pyogranulomas and parasites. The dilated bile ducts contained large amount of *D dendriticum*.

In six of the 9 animals of herd A, eggs of *D dendriticum* were seen; in herd B all the three alpacas showed eggs of *D dendriticum* and in herd C five of the six alpacas exhibited eggs of the parasite in their feaces.

Discussion

The liver lesions seen in this case most likely were due to *D dendriticum* infection. Unfortunately, no analysis (bacteriological or other) was done to confirm that there were no other pathogens involved. Thus the actual cause of death could not be confirmed to be entirely due to the extensive chronic liver lesions, but considering the chronic nature of stress etc may have contributed to liver failure leading to death. Alpacas often hide clinical signs of disease. The relatively good condition of the alpaca agree with the postmortem findings of similar cases in Germany and Switzerland (Wenker *et al*., 2001).

This is the first report of dicrocoeliosis in alpacas in Sweden confirming reports from the European mainland of the presence of this ``new`` parasitosis in alpacas.

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32. Emerging Infectious Diseases in Arabian Camels (*Camelus dromedarius*)

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Introduction

Over the last 15 years, more than 1000 necropsies were performed at CVRL on adult camels as well as the same number on camel calves. Out of this huge number of cases several diseases were discovered for the first time in dromedaries like Rhodococcus-pneumonia, equine rhinitis-A-virus-abortion and Glanders. Other diseases were described in detail for the first time in camels in the Middle East. On the other hand, experimental trials with dromedary and Bactrian camels provided lot of new knowledge on economic important diseases like FMD. Selected diseases are presented in this paper.

Methods and Results

Natural *B. mallei* infections are known to occur in various animal species (e.g. equines wild felines, bears, wolves and dogs). Both species of Old World Camels (OWC), the dromedary (*Camelus dromedarius*) and the Bactrian camel (*Camelus bactrianus*) are susceptible to *Burkholderia (B.) mallei* (glanders) and *B. pseudomallei* (melioidosis) infection (Wernery *et al.*, 1997; Wernery and Kaaden, 2002) and dromedaries have only been artificially infected with *B. mallei* (Samartsev*et al.*, 1940;Curasson, 1947). However, natural infection of camels has not yet been reported. During necropsy of a serological positive dromedary typical glanderous lesions in the lung, choanae and nasal septae were observed. *Burkholderia mallei* was isolated from the nasal pusand confirmed by PCR (Wernery *et al.*, 2011).

Rhodococcus (R). equi, a recognized pathogen in horses (Ainsworth, 1999; Giguère and Prescott, 1997), affects also New World camelids (Hong and Donahue, 1995; Cuteri *et al.*, 2001), but there were no reports of *R. equi* infection in Old World camelids yet. Four cases of disseminated *R. equi* infection in adult breeding dromedaries occurred at one camel farm near Dubai within 16 months of each other (Kinne *et al.*, 2011). At necropsy the lungs were diffusely consolidated with large caseous areas. Histology revealed severe suppurative to necrotising pneumonia with multiple encapsulated abscesses. Immunohistochemistry enabled the detection of 15- to 17-kDa antigens (VapA) of *R. equi* in the lung sections. High numbers of *R. equi* were isolated from the lung lesions as well as from liver, spleen and mediastinal lymph nodes, indicative of septicaemia. The isolated strains were PCR-positive for the specific virulence plasmid (VapA-Gen) of *R. equi*, indicating virulent strains and containing an 85-kb type I plasmid.

Camels are mentioned in OIE list of animals susceptible to FMDV infection, although dromedaries are of low susceptibility to FMDV infection (Wernery *et al.*, 2006). However, FMD-like lesions were described in Bactrian camels in Russia (Orlov, 1963; Terentieva, 1975). During several FMD-outbreaks in Mongolia also Bactrian camels got sick, although the diagnosis was made solely on clinical signs (Sakamoto and Yoshida, 2002). Hence, an experiment was designed to compare susceptibility of dromedary and Bactrian camels to FMDV infection. For this purpose 8 dromedaries and 2 Bactrian camels (all serologically negative against FMDV) were inoculated subepidermo-lingually with FMDV types A and O.

Interestingly, while none of the dromedaries showed a reaction to the FMDV infection, the two inoculated Bactrians developed moderate to severe clinical signs. Elevated rectal temperature of 39.0 to 39.2°C was observed in the 2 Bactrian camels after 1 week (Larska *et al.*, 2008). On the same day animals developed depression, lameness of the hind feet and on the next day local inflammation, swelling and exudation of wounds on the band of footpads were observed. Severe lameness of hind legs, reluctance to walk and stand, pain and lesions developed, and one camel lost the entire epidermis of the footpad sole. After 3 weeks the lesions were healed and the skin of the footpad sole was replaced by new tissue.

Discussion and Conclusion

Glanders and *Rhodococcus equi*-infection were discovered at CVRL for the first time in dromedaries. Here we describe the first reported case of a natural infection with *Burkholderia mallei*

in a dromedary in the course of a glanders outbreak in horses. It is a new emerging disease for camels. Since it is a notifiable disease in equine and a zoonosis, it should be listed as notifiable disease also for camels. Also *R. equi* infection is a new emerging disease for dromedaries. Since adult camels in general do not suffer from bacterial caused pneumonia (except tuberculosis), *R. equi* infection has to be considered in pneumonic cases. Both diseases have a strong link to equids.

Our experimental investigations on FMD confirmed that the dromedary (opposite to the Bactrian camel) is not susceptible to FMD. This fact should be considered in legislation to remove the dromedary from the list of animals susceptible to FMDV infection. In the meantime classical FMD was described by Bold (2012, in press), and the FMDVO also isolated from Bactrian.

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33. Molecular Diagnosis of Camel Diseases

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Introduction

The camel is an important animal, an icon of adaptation to the desert eco-system with unique physiological characteristics. Apart from its use as a source of milk and meat, there is an increased interest in other aspects of camels such as camel racing, which is an important part of the Middle Eastern culture. Thus, continuous monitoring of their health status is imperative.

Infected camels can affect camel herds and induce significant economic losses to the industry. Some of these diseases, such as tuberculosis, brucellosis and West Nile Virus infections are zoonotic and could also pose a serious public health problem. In this region, knowledge of camelid diseases has been limited, as the available diagnostic tests have not been validated for these diseases. Current methods of detection rely on culture, ELISA and histology. These are time consuming and could prolong the time between diagnosis and treatment. Accurate and sensitive diagnostic procedures need to be put into place in order to speed up the diagnosis of the disease.

High levels of abortion in camel herds threaten camel breeding in this region. The main etiological agents responsible for this condition in camels are Brucella and Trypanosoma. Camels are not known to be primary hosts for any of *Brucella* organisms, but they are susceptible to both *B. abortus* and *B. melitensis* (Nicolletti 1989). Trypanosomosis is mainly caused by mechanical transmission of *T. evansi* by biting flies. The overall productivity of a camel herd regarding calves, milk and weight gain is greatly impaired.

In the past eight years, we have put a considerable amount of effort into developing and compiling PCR-based diagnostic tests for camel diseases. This has also been instrumental in screening populations of camel livestock and to identify new strains that can help in tracing infections to their sources. This paper highlights the use of different PCR assays that we have established for screening and detecting existing or emerging camel diseases in the UAE.

Materials and Methods

Primer sequences were taken from published papers or designed using Primer 3 express and ordered from Metabion, Germany. PCR reagents were ordered from Roche. DNA was extracted from the samples using phenol-chloroform method or MagNA Pure automated DNA extraction (Roche). In case of RNA viruses, RNA was extracted from samples using Trizol (Sigma) or Qia Amp viral RNA extraction kit (Qiagen). PCR amplification of DNA was done using specific primers for each pathogen. Real-time PCR assays were performed using Roche light cycler (Manheim, Germany). The assay is carried out using a positive and negative control with each run. PCR cycling conditions for each test were determined empirically or taken from published papers.

Results

The results of the development and application of PCR to diagnose bacterial, viral and protozoan diseases in camels are summarized in Table (1). The routine diagnostic application of the PCR for camel diseases started as early as 2003 and today a total of 16 PCR assays are in routine use for the detection of 2 protozoan, 9 bacterial and 5 viral diseases. This is in addition to development of 20 other PCR assays for detecting pathogens of other animal species.

Table 1: PCR assays developed for pathogen detection in camels

S.No	Test	PCR	Pathogen
1	Chlamydophila	qPCR	Bacteria
2	West Nile Virus	PCR	RNA virus
3	*Trypanosoma evansi*	qPCR	Parasite
4	Mycobacterium spps.	qPCR	Bacteria
5	Mycoplasma spps.	PCR	Bacteria
6	*Burkholderia mallei*	qPCR	Bacteria
7	*Taylorella equigenitalis*	qPCR	Bacteria
8	Foot and mouth disease	RT-PCR	RNA virus

9	Equine Herpes Virus	qPCR	DNA virus
10	*Coxiella burnettii*	qPCR	Bacteria
11	Brucella spps.	qPCR	Bacteria
12	Camelpox	qPCR	DNA virus
13	Piroplasmosis	qPCR	Parasite
14	Adenovirus	PCR	DNA virus
15	*Clostridium perfringens*	PCR	Bacteria
16	African horse sickness	qPCR	RNA virus

We have succeeded in converting most of the classical gel based PCR detection assays in our lab to real time PCR which combines speed with sensitivity. The key feature of real time PCR is that the amplified DNA is detected as the reaction progresses in real time. The presence of these specific DNA sequences in the reaction is detected by an increase in the fluorescence observed from the relevant probe and is reported as a cycle threshold value (Ct) by the real time thermo cycler.

To date, over 700 camel samples have been tested by PCR-based methods of which 250 samples are tested for brucella and 300 samples for trypanosoma. Clostridial diseases are also known to occur sporadically in camels and cause enterotoxaemia (Wernery and Kaaden, 2002). Multiplex real-time PCR detection of clostridium toxin genes is also being developed.

Conclusion

Knowledge of camelid diseases is limited due to unavailability of diagnostic tests in the region. this puts specific constraints on disease control. PCR-based assays are the preferred methods as compared to the traditional methods like culture and ELISA. These assays are quick, reliable, sensitive and specific. They can be used either in conjunction with the traditional methods or as a secondary confirmation.

More research is currently underway to elucidate the role of some of the pathogens mentioned in the epidemiology and pathogenesis of several diseases. Surveillance programs for camel herds especially dairy should be carried out to prevent sudden outbreaks of diseases and PCR-based method could be a reliable and quick method to contribute significantly to this task.

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34. Muscular Sarcosporidiosis of Dromedary Camels (*Camelus dromedarius*) in Mauritania and Chad

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Introduction

The breeding of the dromedary (*Camelus dromedarius*) occupies an important place in Mauritania and Chad because of multiple services offered by this animal (meat, milk, money, transport, social prestige). More and more dromedary's meat belongs to the daily meals in Mauritania and Chad. Consequently, it is important to consider the sanitary security of this food for consumers of which some have a fragile medical condition in particular the pregnant women, the elderly and the children. Among the dromedary affections infecting muscles is sarcosporidiose. The latter can reach variable prevalences from one country to another (Fathy and al., 2009; Kane and al., 2009; Hussein and Warrag, 1985; Hagi and al., 1989; Woldemeskel and Gumi, 2001; Fatani and al., 1996; Valinezhad and al., 2008). Moreover, the sarcosporidiosis of the dromedary is a potentially zoonotic protozoosis (Valinezhad and al., 2008).

In Mauritania, this infection was the subject of a preliminary study (Kane and al., 2009), contrary to Chad. This work aims to determine the prevalence of the sarcocystic infestation in the muscles of the slaughtered dromedaries in the slaughter-houses of Nouakchott (Mauritania) and NDiaména (Chad) and to identify the parasitic species in question.

Material and Methods

The present study was carried out in August 2008 and September 2009 in the slaughter-houses of Nouakchott (Mauritania) and Djaména (Chad). The carcasses of the dromedaries in the two sites were examined in order to detect macroscopic lesions compatible with the sacrosporidiosis. Sampling of muscles was carried out on 58 carcasses at the slaughter-house of Nouakchott and 30 carcasses at the slaughter-house of NDiaména, with a total of 88 animals 3 to 9 years old. On each carcass various types of muscles (heart, diaphragm, neck and tongue) were collected. The samples intended for the histological were fixed in 10% formalin and those intended for parasitologic analysis were kep cold and then conveyed to the laboratories of Histopathology (Beddiya clinic at Nouakchott and EISMV of Dakar) and Parasitology (EISMV of Dakar). The histological exam was carried out according to routine methods of staining with the haematoxylin-eosin stain. The parasitologic analysis was based on the method described by Seneviratna and al. (1975) based on a muscular digestion by pepsin. Only 21 positive samples in microscopic exam were subjected to the parasitologic analysis.

Measurement and photography of the parasitic cysts were carried out in the Imagery Laboratory of EISMV by using the software *LAS EZ version 1.8.0* et *Motic Images Plus 2.0 M.L.* The statistical analysis was made by Excel version 2007 of Microsoft and R *Recommander*. The threshold of significance of the prevalence difference xis fixed at 5% ($P<0,05$).

Results and Discussion

The macroscopic examination of the carcasses did not reveal any macroscopic lesions compatible with the sarcocystic infestation. This result corroborates those of other authors (Hussein and Warrag, 1985; Woldemeskel and Gumi, 2001 and Valinezhad and al., 2008). On the other hand, the histological examination showed average prevalences of 48% and 63% respectively for the slaughter-houses of Nouakchott and Djaména. The prevalence of the sarcocystic infestation appears higher in the slaughter-houses of Djaména than in the slaughter-houses of Nouakchott although the difference is not significant. The prevalence obtained with Nouakchott is higher than those obtained by Kane and al. (2009) in Mauritania (13%) and lower than those obtained by Woldemeskel and Gumi (2001) in Ethiopia (45%). Those of the slaughter-houses of Djaména are similar to the prevalence observed in Egypt (64%) by Fathy and al. (2009). In addition, our results are largely lower than those obtained in other countries by other authors, in particular in Sudan (81%) by Hussein and

Warrag (1986), Saudi Arabia (88%) by Fatani and al. (2001), and in Iran (84%) reported by Valinezhad *et al.* (2008). Thus, the prevalence of the sarcocystic infestation of dromedary differs according to the authors and the study sites. In the various taken muscles, the average prevalence of infestation is, for the slaughter-houses of Nouakchott, 40%, 27%, 23% and 20% respectively in the tongue, the diaphragm, the neck and the heart. At the slaughter-houses of Djamena, for the same muscles, they are in the same order respectively of 35%,16%,14% and 10%. The difference of the infection rate, in these two slaughter-houses, is not statistically significant. From these results, in the different sites, the tongue is more infected followed by diaphragm, whereas the cardiac muscle is less infected. In Ethiopia, Woldemeskel and Gumi (2001) obtained lower rates of infestation than ours in the diaphragm (11,57%) and the heart (9,17%). On the other hand, Valinezhad *et al.* (2008) showed that the heart is the muscle more infested (48%) followed by masseter (46,8%), diaphragm (41,6%) and tongue (28%).

The parasitic cysts measures are: $57,27 \pm 21$ µm (length), $16,43 \pm 8,45$ µm (diameter), and $0,69 \pm 0,20$ µm (wall thickness) at the slaughter-houses of Nouakchott. At the slaughter-houses of Djaména, these dimensions were respectively of $84,62 \pm 20,65$ µm, $19,41 \pm 9,43$ µm and $1,07 \pm 0,92$ µm. Dimensions obtained at Nouakchott are comparable with those obtained ($55,20 \pm 15 \times 21 \pm 7$ µm) by Kane and al. (2009). These dimensions are different from those of *Sarcocystis camelicanis* (72,5 - 264 X 9,9 - 29,5 X 0,5 - 1 µm) and *Sarcocystis. cameli* (73 - 155 X 23 X 29,5 X 2 - 3 µm) found by Manal and al. (2006) to Sudan, and of those by Fatani and al. (1996), in Saudi Arabia. To the slaughter-houses of Djamena, cyst dimensions are comparable with those (72,5-264 X 9,9 - 29,5 X 0,5 - 1 µm) reported by Manal and al. (2006) to Sudan. Thus, Sarcocystis species found in Chad are similar of those in Sudan. However, complementary studies are necessary with more specific methods such the PCR and the immunofluorescence test in order to identify more specifically the parasitic species. Moreover, it was noted inflammatory lesions like eosinophilic myositis. These types of lesions were reported by Kane *et al.* (2009) in Mauritania and Valinezhad *et al.* (2008) in Iran.

By enzymatic digestion, the following prevalence is obtained in different muscles: heart (67%), diaphragm (33%), tongue (27%) and neck (0%). This confirms microscopic results by highlighting the Sarcocystic bradyzoïtes. These bradyzoites measured $18,55 \pm 3,65 \times 4,5 \pm 0,61$ µm. These dimensions are higher than those reported ($15,35 \pm 0,29 \times 4,1 \pm 0,26$ µm) by Fatani *et al.* (1996) in Saudi Arabia by using trypsin.

Conclusion

The muscular sarcosporidiosis has a considerable rate infection among slaughtered dromedaries examined in Mauritania (48%) and in Chad (63%). This relatively high rate must challenge all the actors of camel channel in the two countries in order to undertake actions for better considering this parasitic infestation to control it. These actions must include, among other things, research, capacities reinforcement of the animal health professionals, and the sensitizing of the breeders and the consumers.

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35. Subclinical Goiter in Camels *(Camelus dromedarius)* in the Dhofar Region of Oman

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Introduction

The thyroid gland is important in maintaining health, and iodine is a crucial constituent of thyroid hormone (Arthur and Beckett, 1999). Nowadays about 800 million people are affected by iodine deficiency disorders that include goiter, (Triggiani *et al*, 2009). Goiter is well documented in many animal species (McDowell *et al*, 1983). Low iodine uptake of dromedary camels predispose them to iodine deficiency than other domestic animals (Abdel-Wahab and Osman, 1971). Data on goiter in dromedary camels are scarce. Apart from the reports of colloid goiter (Hruska and McDermid, 1979 and Tageldin *et al*, 1985) and clinical and subclinical colloid goiter (Abu-Damer *et al*, 1990) no reports could be traced in the literature.

The aim of this study was to assess subclinical goiter in dromedary camels raised in mountainous areas of the Sultanate of Oman.

Materials and Method

Blood was collected in EDTA, heparin and plain tubes from 52 camels of different ages. Bilobed thyroid glands were collected from apparently healthy slaughtered dromedary camels at the central slaughterhouse, Salalah, Oman. The age of the animals were ranging from 1-20 years. Both glands were weighted. Representative portions from each pair of thyroids were fixed in 10% buffered formalin, processed, sectioned and stains with H&E. Selected slides were subjected to immunohistochemistry using HBME1, GAL9, Calcitonin and CD56 as tumor markers. The marker pattern and intensity were recorded.

Hematological parameters were determined. Assessment of thyroid function was carried out by estimation of thyroxine (T4), tri-iodothyronine (T3) and thyroid stimulating hormones (TSH) were determined. Selenium and Vitamin E will be determined. Serum cholesterol, triglycerides, total proteins, HDL cholesterol, GOT, GPT, CK and GGT will be measured.

Results

Based on macroscopic and microscopic pictures, the samples can be divided into four groups: Group1, 42.3% (22/52) showed early stages of colloid goiter characterized by enlargement of thyroid follicles at the periphery which were distended by colloid.

Group11, 28.8% (15/52) represent advanced stages of colloid goiter where both follicles at the periphery and central were enlarged and markedly distended with colloid. 80% (12/15) of this group showed enlargement of the glands and a mixture of macro-follicles and micro-follicles in a form of nodules all over the surface and cut surface which exuded a sticky jelly like fluid (nodular goiter).

Group 111, 15.4% (8/52) showed advanced stages of colloid goiter associated with hyperplastic goiter, the proliferation of the epithelium resulted in reduction or complete occlusion of follicular lumin. 62 (5/8) of this group exhibited enlargement of the glands and macroscopic follicles (nodular goiter).

Group1V, 13.5% (7/52) represent advanced stages of colloid goiter associated with hyperplastic goiter and adenoma, a benign encapsulated tumor with follicles surrounded by thick fibrous capsule, sharply separated from the surrounded tissues.

In advanced stages of colloid goiter papillary projections and thickening of inter follicular connective tissue was observed in few cases. The thyroid weights were ranging between 21.i and 67.2 grams (mean 34. grams).

The immunohistochemistry parameters were negative for carcinoma. Hematological and chemical parameters are in progress.

Discussion

Goiter is defined as non-inflammatory and non-neoplastic enlargement of thyroid gland (Doige and McLaughlin, 1981). Goiter is generally and endemically present in mountainous areas (Tageldin *et al*, 1985). Subclinical goiter is more prevalent in camels at Dhofar area than has been suspected. Colloid goiter is believed to represent an involutionary phase of hyperplastic goiter (Doige and McLaughlin, 1981). Hyperplastic goiter and adenomas had not been reported in the previous investigations (Tageldin *et al*, 1985 and Abu-Damer *et al*, 1990). However, distinction between hyperplastic nodule and follicular adenoma is not so strict (Baloch and LiVolsi, 2002). The carcasses of affected subjects were in good condition and passed for human consumption.

It can be concluded that subclinical colloid goiter is the most common type of goiter. It is either alone or associated with hyperplastic goiter and/or adenomas. Recommendations can be made upon completion of the work and analysis of the parameters.

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36. Mycoplasmosis – A New Disease in Camelids

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Introduction

Classification of certain bacteria families has dramatically changed over the last decade mainly based on the development of molecular techniques, comparing nucleotide sequences of the genome, in particular the 16S rRNA gene sequences; these changes refer also to the mycoplasma family. Bacteria, formerly known as *Haemobartonella* and *Eperythrozoon* species of the order *Rickettsiales* have been re-classified as belonging to the *Mycoplasmataceae* (Table 1). They are named haemotrophic mycoplasmas.

Table 1: Mycoplasmataceae of veterinary importance

Family	Host	Genus (No. of species)	Important species (many more have been identified)
Mycoplasmatace	Cattle, sheep, goats	Mycoplasma (12)	*M.mycoides subsp. Mycoides (2x), M. agalactiae M. mycoides subsp. capri, M. bovis*
		Acheloplasma (1) Ureaplasma (1)	*M. capricolum subsp. capripneumoniae, M. conjunctivae* *M. capricolum subsp. capricolum, M. ovis* *M. sp.bovine group 7*
	Horses	Mycoplasma (11) Acheloplasma (8)	*M. felis* *M. equirhinis*
	Dogs and cats	Mycoplasma (15)	*M. canis, M. haemofelis (Haemobartonella felis)* *M. cynos, M. gatae*
		Acheloplasma (1) Ureaplasma	*M. felis*
	Swine	Mycoplasma (13)	*M. hyopneumoniae, M. suis (Eperythrozoon suis)* *M. hyorhinis*
		Acheloplasma (5) Ureaplasma (1)	*M. hyosynoviae*
	Camelids	**Mycoplasma (2)** **Acheloplasma (2)** **Ureaplasma (?)**	***M. haemolamae (Haemobartonella spp.)*** ***M. arginini*** ***A. laidlawii, A. oculi***
	Domestic birds	Mycoplasma(17) Acheloplasma(2) Ureaplasma(2)	*M. gallisepticum* *M. meleagridis* *M. synoviae*

Results

The presentation is divided into 3 parts. The first part deals with haemotrophic mycoplasmas, the second part with ―classical‖ mycoplasmas and the third with a mycoplasma – outbreak in dromedaries in Iran.

Haemotrophic mycoplasmas are now a well known bacteria group in the USA in NWCs but also in Europe where more and more SACs are kept. Scientists have also described a double infection in an alpaca with haemotropic mycoplasma and anaplasma.

Mycoplasmosis has frequently been identified in young llamas (McLaughlin *et al*., 1990; Semrad, 1994). Such llamas have a history of weight loss and stunted growth and development of acute or recurrent infectious conditions. During necropsy, severe fibrinous polyserositis involving the thoracic and abdominal organs, moderate diffuse non-suppurative interstitial pneumonia, splenic hyperplasia, necrotizing enteritis, widespread vascular thrombosis and anaemic infarcts in the liver are

observed. These organisms are attached to the surface of red blood cells of the affected llamas and are often found in clusters, usually towards the edge of the cell (Wernery *et al*., 1999). Also double infections with *A. phagocytophilum* and *M. haemolamae* have been described in SACs (Lascola *et al*., 2009). They are extremely difficult to differentiate from anaplasma of which several species also parasitize in red blood cells, when blood smears are checked.

Much progress has been made in the study of the haemotrophic mycoplasmas in camelids, and diagnostic testing has been greatly improved over the last few years. A PCR-based assay has been developed made available for diagnostic testing by the Veterinary Diagnostic Laboratory at Oregon State University's College of Veterinary Medicine (Tornquist, 2006, 2008). Specifity for *M. haemolamae* was shown by failure to identify other than mycoplasma species like *M. haemosuis, M.haemofelis,* and *M. genitalium*. All studies have elucidated, that many infections are subclinical, and that clinical signs of infections with these organisms can vary widely. Clinical infections are associated with fever, mild to marked anaemia, depression, icterus, infertility, oedema, poor growth rate and mild to severe hypoglycaemia. It is not yet investigated, if these bacteriae may cause or serve as co-factors in some forms of immune suppression.

Beside the haemotropic mycoplasmas, ―classical‖ mycoplasmas have been investigated in camelids.

In spring 2011, a severe respiratory disease occurred in Iran, Pakistan and Afghanistan affecting several thousand dromedaries with high mortality. Several promed reports were released. From Iran, CVRL received blood and nasal swabs from diseased animals. *Mycoplasma spp.* were isolated from several swabs. The results of this investigation are reported during the presentation.

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37. Ticks of Camels (*Camelus dromedarius*) in Oman

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Introduction

The Arabian camel (*Camelus dromedarius*) is one of the principal sources of meat and milk in Oman. In spite of this, very little is known about the health problems of this animal in the country. Ticks, and the diseases that they transmit, are among the most important causes of morbidity among domestic animals and if not controlled appropriately, they limit production in livestock farming (Howell *et al.* 1978). In Oman, few studies have been conducted to determine the species of ticks that infest camels in the country. According to the studies conducted by Hoogstraal, 1980; Papadopoulos *et al.* 1991; and Wassef *et al.* 1997, six species of ixodid ticks and one species of argasid tick were found in association with camels. The species of ticks and the regions of the country in which they were found were as follows: *Amblyomma variegatum* (Dhofar Region), *Hyalomma anatolicum* (Northern and Central regions), *Hyalomma dromedarii* (Northern, Central and Dhofar regions and Masira Island), *Hyalomma impeltatum* (Northern and Central regions), *Hyalomma marginatum* (Central region), *Rhipicephalus turanicus* (Dhofar Region), and *Ornithodoros savignyi* (Central region).

Since 1997, there has been no further investigation of the ticks that could be infesting camels in the country. To address this paucity of information, a survey was carried out to determine the species of ticks that infest camels in the Dhofar Region which is home to more than 45% of the camels in the country (Ministry of Agriculture, Oman 2011).

Materials and Methods

In June 2009, ticks were collected from camels at six locations in Dhofar Region, namely, Muqrah, Ruwiya, Shihat, Kizet, Wadi Heno and Sadah. The geographic coordinates of each location where ticks were collected were recorded at the time of tick collection and later mapped. At each location, at least 10 animals with visible tick infestation were selected for tick collection. Where there were less than 10 infested animals all the infested animals were selected. Ticks were collected from all parts of each animal's body, including the ears, chest, ventral abdomen, limbs, tail and peri-anal region.

Collected ticks were identified and counted using a stereoscopic microscope. Ticks were identified morphologically using keys from various publications, namely Hoogstraal *et al.* (1981), Walker *et al.* (2003), Apanaskevich & Horak, (2005, 2008 and 2009) and Apanaskevich *et al.* (2008).

Results

The ages of the camels from which ticks were collected ranged from two weeks to 18 years. There were 5 males and 42 she camels. A total of 644 ticks collected from the 47 camels consisted of the following seven ixodid species, *Amblyomma (A) variegatum*, *Hyalomma (H) anatolicum*, *H. dromedarii*, *H. impeltatum*, *H. marginatum*, *H.* rufipes and *Rhipicephalus (R) camicasi* (Table 1).

H. dromedarii was the most numerous tick species followed by *H. impeltatum*; while *H. anatolicum* was represented by only one specimen (Table. 1). *H. dromedarii* and *H. impeltatum* were also the most widely distributed species, being found at all the six locations (Table.1). *H. anatolicum* and *R. camicasi* were collected from one location each. Adult and nymphs of *H. dromedarii* were found on infested animals while only adults of the other tick species were found.

Thirty-three of the camels (70.2%) were infested by more than one species of tick. Infestation of individual camels with two, three and four species of ticks were found on 42.6%, 21.3% and 6.4% of the camels respectively

Table 1: Tick species and their numbers collected from camels at different locations in Dhofar Region.

Locations	Elevation*	No. of animals	Amblyomma variegatum		Hyalomma anatolicum		Hyalomma dromedarii			Hyalomma impeltatum		Hyalomma marginatum		Hyalomma rufipes		Rhipicephalus camicasi	
			Adults		Adults		N	Adults		Adults		Adults		Adults		Adults	
			F	M	F	M		F	M	F	M	F	M	F	M	F	M
Muqrah	976	7		5			13	38	45	4	6			1			
Ruwiya	607	10				1	54	9	60	13	16	1	2	3	14		
Shihat	535	3		2				3	13	3	1						
Kizet	430	12	15	41			30	17	36	9	8			1			
Wadi Heno	64	2					5	8	17	9	9						
Sadah	32	13					1	31	72	13	4			1	5	4	1

*Meters above sea level, N= Nymph, F= Female, M=Male

Discussion

The finding of *Hyalomma (H) rufipes* and *Rhipicephalus (R) camicasi* on camels are new host records for Oman. Though both tick species are being reported for the first time in Dhofar region, they have been found on camels in Yemen (Wassef *et al.* 1997). Also *H. rufipes* has been found on camels in Saudi Arabia (Hoogstraal *et al.* 1981), Kuwait and Qatar (Wassef *et al.* 1997).

Of the five other ticks species found on camels in this study, namely *A. variegatum*, *H. anatolicum, H. dromedarii, H. impeltatum*, and *H. marginatum*, two species, H. *dromedarii* and *A. variegatum*,have earlier been reported from Dhofar region (Hoogstraal 1980; Wassef *et al.* 1997). The finding of the other three tick species on camels represents new host records for Dhofar region.

H. anatolicum and *H. impeltatum* have been collected from camels in northern and central regions of Oman (Papadopoulos *et al.* 1991; Wassef *et al.* 1997) but only from the ground in Dhofar Region (Wassef *et al.* 1997). These tick species have been collected off camels in Saudi Arabia (Hoogstraal *et al.* 1981), Yemen (Ueckermann *et al.* 2006) and Qatar (Wassef *et al.* 1997.

There has been only one report of the occurrence of *H. marginatum* on camels in the Arabian Peninsula and that was from the central region of Oman (Papadopoulos *et al.* 1991). The tick species seems to be rare in the Arabian Peninsula. In fact, in this study, only three ticks were found and at only one location.

This study has resulted in an increase in the number of species of ixodid ticks associated with camels in Oman; from six to eight.

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38. Bacterial Camel Mastitis in the Kingdom of Bahrain

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Introduction

The one-humped camel *(Camelus dromedarius)* inhabits the arid and semi-arid areas of Africa. It is a better provider of food in these regions than other ruminants which are severely affected by feed and water scarcity. In the past camels were used mainly for transport while milk, meat, wool and hides were by-products. The search for food, particularly animal protein for the largely increasing human population in developing countries (where the lands are barren, degraded and marginal) has focused on the milk and meat potentials of desert adapted animals (Elamin, F.M. and Wilox, C.J.(1992), Knoss K.H. (1982), Wardeh, M.F.(1994). Reports of inflammation of the camel udder have appeared from various countries, such as Egypt (Hassanein, A. *et al* (1984) and Mostafa, A.S, *et al*. (1987), India (Kapur,M.P., *et al*. (1982), Saudi Arabia (Barbour,E.K., *et al*. (1985),and Hafez, A.M., *et al*. (1987), Somalia (Abdelrahman,OASH,*et al*. (1991), and Arush, M.A. (1984), Sudan (Obeid, A.I. *et al*.(1983) and UAE (Quandil, S.S., and Qudar, J.)

Mastitis is the inflammation of the udder characterized by pathological alterations in the mammary tissues, compositional changes in milk, elevated somatic cells, and pain to the affected animal (Tibary, A. and Anouassi, A. (2000). Mammary gland function is also important for the health and growth of the newborn animal since other diseases are known to have a negative effect on both factors and can cause public health hazards for populations consuming camel milk (Knoess,K.H. (1986). Mastitis takes two forms: clinical mastitis, which is recognized by abnormal milk, signs of udder infection and detection of mastitis pathogens by microbiological culture, and subclinical mastitis were clinical signs are invisible and require indirect means of diagnosis. Evidence indicates that subclinical mastitis causes suffering of the animal, reduces milk yield alter milk properties, impairs preservation and processing and is public health concern for consumers of camel milk (Tibary,A. and Anouassi, A., 2000). In 1987, Ramadan, R.O., *et al* reported chronic unilateral mastitis in 2 female camels. There are divergent opinions as to which bacteria are potentially the primary causal organisms of infectious mastitis in the camel. Barbour *et al* (1985) views Micrococcus spp. as an important causative agent of mastitis whereby (Obeid, A.I. *et al*. (1983)) did not consider this bacterium pathologically relevant. In 1996, Obeid *et al* found Streptococcus spp., Staphylococcus spp., Micrococcus spp., Aerobacter spp. and E. coli to be the main bacterial species causing mastitis. Al Ani , F.K.,and Al-Shareefi, M.R.(1998) found that Streptococcus aureus and Corynebacterium pyogenes were the main cause s of chronic mastitis in Iraq, whereas Streptococcus epidermidis spp., Pasteurella hemolytica, E. coli and Micrococcus spp. were responsible for subclinical mastitis.

From the multitude of bacteria isolated from mastitic milk samples of camel, *Staphylococcus* aureus, Pasteurella hemolytica, and *Staphylococcus* Spp. were found most frequently. Numerous authors believe them to be the primary causative organisms in the pathogenesis of mastitis in the camel (Barbour,E.K.,*et al*. (1985), Hafez, A.M., *et al*. (1987), and Ramadan,R.O., *et al*.(1987). Younan *et al* (2001) also isolated *Streptococcus agalactiae* from three cases with mastitis.

Materials and Methods

In Bahrain camel mastitis is rare. During a period of 5 years only 25 cases were recorded.The teats of camels were cleaned thoroughly and dried; the teat tips were disinfected using disposable paper towel immersed in 70% ethyl alcohol.

The first three streams of milk were allowed to flow out.Approximately 10 ml of milk samples were aseptically collected from lactating camels suffering from clinical and subclinical mastitis in sterile containers for bacteriological examination. Milk samples were examined for any change in their color or consistency.

Bacteriological Examination

A loopful of milk was streaked onto sheep blood agar and MacConkey agar (Difco Laboratories). The plates were aerobically and anaerobically incubated at 37ºC for 24h and a further 24 hours for those plates that showed scanty or no growth. Plates were examined for growth,

morphological features, and hemolytic characteristics. Plates were considered culture negative if there was no bacterial growth on the medium within 72 hours.

Identification of bacteria was made on the basis of colony morphological features, Gram stain, reaction, hemolytic characteristics and catalase test. Isolated bacteria were identifiedusing the API system (API bio Merieux).

Results and Discussion

In terms of decreasing prevalence, the isolated bacteria can be ranked in the following order: Staphylococcus spp. (32%), Corynebacterium pyogenes (20%), Streptococcus spp. (16%), Escherichia coli (12%), Peptostreptococcus spp., Pasteurella hemolytica, Enterobacter spp., and Aeromonas spp. 4% each (Table1). The efficacy of various antibiotics on bacterial isolates from camel milk samples is shown in Table 2.

Treatment

Different antibiotics were used to treat the infection. Table 2 shows the sensitivity of the isolated bacteria to different antibiotics. Most of the isolated bacteria were sensitive to Amikacin and Norfloxacin. Less sensitivity was shown to Azithromycin and Rifampicin. Sensitivity to Streptomycin Neomycin was low.

Table 1: bacteria isolated from mastitis cases in female camels

ISOLATED BACTERIA	NO OF CASES	PERCENTAGE
Stphylococcus aureus	24	24%
Corynebacterium pyogenes	20	20%
Echerichia coli	12	12%
Streptococcus agalactiae	8	8%
Streptococus dysgalatiae	8	8%
Staphylococcus hemolyticus	4	4%
Staphylococcus hominis	4	4%
Proteus mirabilis	4	4%
Micrococcus species	4	4%
Pasteurella aeroginosa	4	4%
Enterobacter species	4	4%
Aeromonas species	4	4%
TOTAL NO. OF CASES & %	100	100%

Table 2: Degree of sensitivity of isolated bacteria to antibiotics

ISOLATED BSCTERIA	AK	NOR	AZM	RD	N	S
Staphylococcus aureus	++	++	++	+	+	+
Corynebacterium pyogenes	++	++	++	++	+	+
E. coli	++	++	++	+	+	+
Streptococcus agalactiae	++	++	+	++	+	
Streptococcus dysgalactiae	++	++	+	++	+	+
Staphylococcus hemolyticus	++	++	++	+	+	+
Staphylococcus hominis	+	+	++	++	+	+
Proteus mirabilis	++	++	++	+	++	++
Micrococcus species	++	++	+	++	++	+
Paseurella aeroginosa	++	++	+	+	+	+
Aeromonas species	++	++	++	+	+	+
TOTAL NO. OF CASES & %						

N.B 9 AK= AMIKACIN , NOR = NORFLOXACIN , AZM = AZITHROMYCIN
RD = RIFAMPICIN , N = NEOMYCIN, S = STREPTOMYCIN

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