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CHARACTERIZATION OF C*amelus dromedarius* IN ETHIOPIA: PRODUCTION SYSTEMS, REPRODUCTIVE PERFORMANCES AND INFERTILITY PROBLEMS

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March, 2014

Debre Zeit

CHARACTERIZATION OF C*amelus dromedarius* IN ETHIOPIA: PRODUCTION SYSTEMS, REPRODUCTIVE PERFORMANCES AND INFERTILITY PROBLEMS

A dissertation submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the Doctor of Philosophy in Veterinary Obstetrics and Gynecology

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Title: Characterization of C*amelus dromedarius* in Ethiopia: Production Systems, Reproductive Performances and Infertility Problems

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Dedication

This PhD dissertation is dedicate to Afar, Somali and Borana pastoralists, who have been straggling with nature to make life meaningful in the unexpected extremely harsh environment

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF APPENDIXES

TABLE OF CONTENT **PAGE**

TABLE OF CONTENT (Continued)

TABLE OF CONTENT (Continued)

LIST OF ABBREVIATIONS

AAU	Addis Ababa University
AFC	Age at First Calving
AFS	Age at First Service
AGID	Agar Gel Immunnodiffusion
AINOV	Aino Virus
AKAV	Akabane Virus
ANRS	Afar National Regional State
BLV	Bovine Leukemia Virus
BoHV	Bovine Herpes Virus
BPIV	Bovine Parainfluenza Virus
BPSV	Bovine Popular Stomatitis virus
BVD	Bovine Viral Diarrhea
CHUV	Chuzan Virus
CI	Calving Interval
CPE	Cytopathic Effect
CSA	Central Statistics Agency
DNA	Deoxyribonucleic Acid
EMB	Eosin Methylene Blue
FAO	Food and Agriculture Organization

LIST OF ABBREVIATIONS (Continued)

FBM	Fetal Bovine Muscle
FCS	Fetal Calf Serum
FLK	Fetal Lamb Kidney
GETV	Getah Virus
HmLu	Hamster Lung
IBAV	Ibaraki Virus
ICSI	Intra-cytoplasm Sperm Injection
IMViC	Indole, Methyl red, Voges-Proskauer and Citrate
IPS	Industrial Projects Service
IUCN	International Union for Conservation of Nature and Natural Resources
JEV	Japanese Encephalitis Virus
JZAO	Jijiga Zone Agricultural Office
MDBK	Madin Darby Bovine Kidney
MEM	Minimum Essential Medium
MGG	May Grunwald Giemsa
MoARD	Ministry of Agriculture and Rural Development
NAHDIC	National Animal Health Diagnostic and Investigation Center
OIE	World Organization for Animal Health

LIST OF ABBREVIATIONS (Continued)

PADS	Pastoral Areas Development Study
PhD	Doctor of Philosophy
SCF-UK	Save the Children Fund - United Kingdom
TCID	Tissue Culture Infective Dose
TFNA	Testicular Fine Needle Aspiration
TFNA	Testicular Fine Needle Aspiration
TPB	Triptose Phosphate Broth
USA	United States of America
USD	United States Dollar
VD	Vagues Prousker
VMTH	Veterinary Medical Teaching Hospital
VN	Virus Neutralization
XLD	Xylose-Lysine-Desoxycholate

CHAPTER ONE

1. LITERATURE REVIEW

The taxonomic distinction, evolution and current distribution of *Camelidae* family are going to be treated for highlighting. Special focus is directed to dromedary camels in the peculiar adaptive mechanisms, challenges of camel production and future prospects are concerned.

1.1. Taxonomic Classification, Evolution and Distribution of *Camelidae*

The *Camelidae* are very distinct from other families in it includes the Bactrian Camel (*Camelus bactrianus*), the Dromedary (*Camelus dromedarius*), Guanaco (*Lama guanicoe*), Llama (*Lama glama*), Viçuna (*Vicugna vicugna*), and Alpaca (*Vicugna pacos*) (Franklin, 2011) as indicated in figure 1. Around 95% of the camelids in the Old World are Dromedaries, with nearly all of the remaining 5% being domestic Bactrian Camels (there are no more than a few thousand wild Bactrian Camels). In the New World, around 47% of camelids are Llamas, 41% Alpacas, 8% Guanacos, and 4% Viçunas (Franklin 2011).

A

B

Figure 1: *Camelidae* family of the world (A) old world camelids and (B) new world camelids from Wikimedia Commons

After the New anatomical and DNA evidence on the relationship between Artiodactyla (even-toed ungulates like *Camelids*) and *Cetacea* (whales and dolphins) recently led to a merging of the two orders into a new group, *Cetartiodactyla* as reviewed by Kulemzina, (2009). Following this findings, experts had not agreed on whether to define *Cetartiodactyla* as an official taxonomic order that would replace *Artiodactyla* and *Cetacea*. Some continue to list camels in the order *Artiodactyla* (Franklin, 2011) or use the term *Cetartiodactyla* without defining it as an order (IUCN, 2008).

Camelids originated in North America during Eocene, 40-45 million years ago and become extinct later from their original places (Cui *et al*., 2007). Camels that would give rise to the dromedary and Bactrian camel spread into Asia, across Europe, and as far west as Spain (Figure 2). *Camelids* would give rise to the current day South American species spread south across the Panamanian Land Bridge. Because of their ability to thrive under tough conditions of extreme temperature and scarce food and water, domesticated *Camelids* have been extremely important to the development of human cultures in the steppes of Eurasia, the deserts of Africa, and the arid Andes of South America. Earliest *Camelids* were similar to modern guanaco but rabbit-sized (30 cm at shoulder). All *Camelids* are diurnal and are adapted to harsh and dry climates and are all highly social (Franklin, 2011).

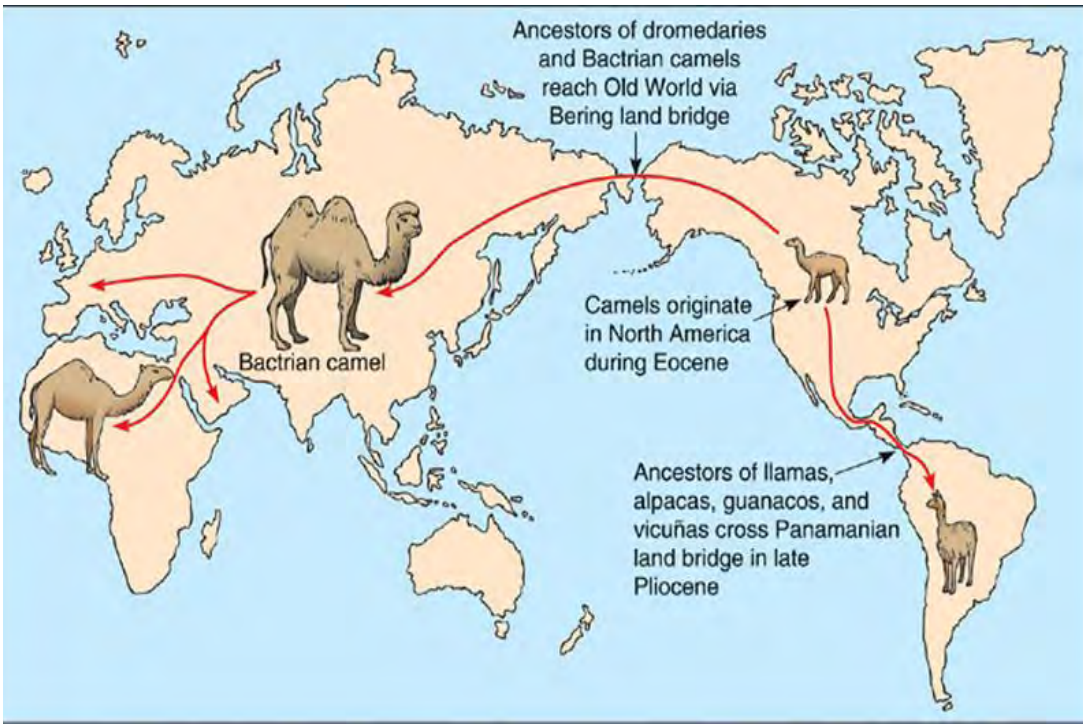


Figure 2: Migration of *Camelidae* from its original place in Alaska from Wikimedia Commons

There is unclear evolutionary relationship between *C. bactrianus*, *C. dromedarius*, and *C. ferus*. However, DNA studies show wild Bactrian camel is not the ancestor of two domesticated species as previously thought and *C. ferus* is separate lineage and not direct progenitor of *C. bactrianus* (Ji *et al*., 2009, Silbermayr, 2009) but previously, they were considered to be a sub-species, *C. bactrianus ferus* (Grubb, 2005). Dromedary camels, Bactrian camels, llamas, and alpacas all have 74 chromosomes (2x36 = 72 autosomes and one pair of sex chromosomes), and they are phylogenetically related to each other while the wild camel has 77 chromosomes (Potts 2004; Hare, 2008). Similarly, there is no plausible evidence to confirm Bactrian camels as ancestor of dromedaries (Kinne *et al*, 2010). According to FAO (2008) report, currently there are about 97 dromedary and 16 Bactrian camel breeds all over the world.

Dromedary (*Camelus dromedarius*) also named as one-humped/Arabian camel is associated with East and northern Africa as well as southwestern Asia. It belongs to the domain *Eukarya,* kingdom of *Animalia,* phylum of Chordate, class Mammalia, order Artiodactyla, family *Camelidae*, Genus Camelus and species of dromedarius. This camel is entirely domesticated (except for a free-ranging feral population in central Australia introduced in the late 1800s). The Dromedary was domesticated around 4000-5000 years ago; the wild form is believed to have gone extinct by 2000-5000 years ago. In addition to their natural migration routes, archeological finds indicate that both the dromedary and domestic Bactrian camel were imported into Northern provinces of the Roman Empire for military and civilian use (Pigière & Henrotay, 2012).

The Food and Agriculture Organization (FAO) estimates the total population of camel in the world today to be 25.89 million, of which 89% are one-humped dromedary camels and the remaining 11% are the two-humped (*Camelus bactrianus*) generally found in the cold deserts of Asia. Over 80% of the world's camel population is found in Africa with the highest concentration in North East Africa which accounts for 63% of the world camel population. Ethiopia is estimated to be the third largest camel herd in the world after Somalia, and Sudan (FAO, 2013).

1.2. Special Adaptive Features of *Camelus dromedarius* to the Desert Environment

Dromedary camels are known by their peculiarity features and in this section the main special anatomy, physiology and behavioral adaptation mechanisms helping them to live with the hostile desert environment are entertained in brief and comprehensive manner. Series of distinctive anatomical and physiological adaptations help them to survive a long period of time without feed and water.

Dromedary camels are hardy desert animal are famous for their exceptional tolerance of heat and deprivation of water or feed in arid climate (Farah *et al.* 2004). The remarkable survival and performance in the desert attribute results from a very low basal metabolism and exceptional water-conserving adaptations.

The camels' thick coats insulate them from the intense heat radiated from desert sand and during the summer the coat becomes lighter in color, reflecting light as well as helping avoid sunburn. Dromedaries have a pad of thick tissue over the sternum called the pedestal. When the animal lies down, the pedestal and other small areas of padded contact points on with the ground their legs raise the body from the hot surface and allow cooling air to pass under the body (Figure 3). As behavioral adaptation to the hot sand they face towards the sun while resting on the desert sand in a stream lined state to minimize the exposed parts of their body to the sun (Figure 4).

Figure 3: Anatomical adaptation mechanism of dromedary camels in the hot desert sandy environment (Photo by Simenew Keskes, 2011)

Figure 4: Behavioral adaptation mechanism of dromedary camels facing the sun to reduce exposure of their body (Photo by Simenew Keskes 2011)

The long legs they have are typically useful for them to keep far high from the hot sand in the desert and allow cold air to move in the under parts of the belly as cooling mechanism. Their large broad 'elastic' pads with two finger nail-like toenails on front are also important structures to easily walk on the desert sand which is not possible for other ungulates walk on tips of hoof-covered toes. The advantage of this broad leathery pad in camels is to disperse their weight in a wider surface area and their feet don't sink in the loose sandy soil.

Hump is another anatomical structure composed of fibrous tissue and fat but not water reservoir at all. The size and shape of the hump signifies availability of nutrition almost disappears during starvation. When the animal is well fed, the hump is plump and erect, but in starved animals it shrinks or flops to one side. The large fat reservoir in the hump sustains camels through times of food scarcity. When the fat is metabolized, it acts as a source of energy and water as every kilo gram of fat mobilization produces one milliliter of water. The other advantage of hump in the dromedary camel is to concentrate body fat in small area and minimizes its presence throughout the rest of body which indirectly reduces heat-trapping that occurs with insulating layers of fat in the hot climate.

The Slit-like closable nostril protects against blowing sand and moistens air on its way to the lungs. When camel exhales water vapors becomes trapped in their nostrils and is reabsorbed into the body to conserve water. Their eyes are also equipped with protective bony arch and long eyelashes to safeguard from sun shield and frequent blowing sand in the desert respectively. If sand gets lodged in their eyes, they can dislodge it using their transparent third eyelid (nictitating membrane) (Butler and William, 2005). Split upper lip assists feed selection and easy prehension during browsing and their mouths have a thick leathery lining, allowing them to chew thorny desert plants. The small wound ears covered with tufts of hair is protected from entering of the blowing sand.

The oval red blood cells in dromedary camels can easily flow quicker in a dehydrated state of the animal as compared to the round shaped red blood cells in other mammals. These red blood cells are also enormously expansible. In sections they appear similar to sickle cells of humans. In view of the peculiar nature of the red blood cells of the *Camelidae*, the study by Warda & Zeisig (2000) is especially interesting. They examined the composition of the dromedary red cell membranes. There was 28.8% sphingomyelin, 12% phophatidylcholine, and phosphatidylserine

each. Because of the shorter and less saturated fatty acid chains that they identified, the dromedary red cell membranes are more fluid than those of human red cells and perhaps this explains the remarkable stretching ability in camels. The ellipsoid shape of camel erythrocytes is very stable and that the cytoskeleton differs from that of human red cells and they may expand with distilled water to 400% before they rupture (Omorphos *et al*., 1989).

The digestive and urinary tracts are well specialized in water conservation. Cattle lose 20 to 40 liters of fluid daily through feces, whereas camels lose only 1.3 liters. This is one of the primary methods for resisting water deprivation in the desert. Fluid is absorbed in the end part of the intestines, where the small fecal balls are produced (Breulmann *et al*., 2007). The long loops of Henle, which are four to six times longer than in cattle, have the function of both, concentrating urine and reducing its flow. A dehydrated camel urinates only drops of concentrated urine being shown by white stripes of salt crystals on the hind legs and tail. This concentrated urine not only serves to conserve water, but also allows camels to drink water which is more concentrated than sea water (above 3% NaCl), and to eat salty plants (halophytes) that would otherwise be toxic. The body of camels can tolerate loss of water over 30% of body weight whereas most mammals die if they lose half of this value (Franklin, 2011). As a means of water conservation strategy, dromedary camels have very few (25%) of number of sweat glands in cattle and they can only start to sweat at very high temperature.

The large camel nasal surface absorbs the vapor and cools a network of small blood vessels, named the ‘carotid rate’. This carotid vessel network surrounds the jugular vein and cools its blood. On the way to the heart the cooled venous blood meets the warm arterial blood going to the brain and eyes, cooling it by more than 4 ºC. .Brain cooling in camels at their rete mirabile, a complex of veins lying very close to the carotid artery utilizes countercurrent blood flow to cool blood flowing to the brain

and eyes. This protects their brain from heat damage as it is the first organ affected by high temperature (Schmidt-Nielsen, 1998).

1.3. Importance of Camel as Multipurpose Animal over Other Livestock Species

Camelids have played critical roles in a range of human societies. multi-purpose animal and unlike any other domesticated animal, has been utilized by humans for centuries for transport, traction power, milk, meat, wool, skin and even fuel. In the hot deserts of East and North Africa, dromedaries have served as beasts of burden and provided meat, milk, fat, and fuel, as well as soft wool for clothing and tents. In some areas of northern Kenya, live dromedaries are bled for consumption of the blood. The main economic value of camels is derived from their milk, meat, use in transportation, and for riding in sports (racing), tourism or corps. In the case of tribal feuds, camels are the only means of payment of blood money to the lineage of the deceased (Hussein, 1993). Pastoralists see camels as a banking system or security against drought, disease, and the other natural calamities. In general terms however, milk and meat dominate the overall value derived from camels (Muli *et al*., 2008). In Ethiopia as typical example the percentage contribution of meat, milk, transport and other services are indicated in figure 5 in relation to sex of camels.

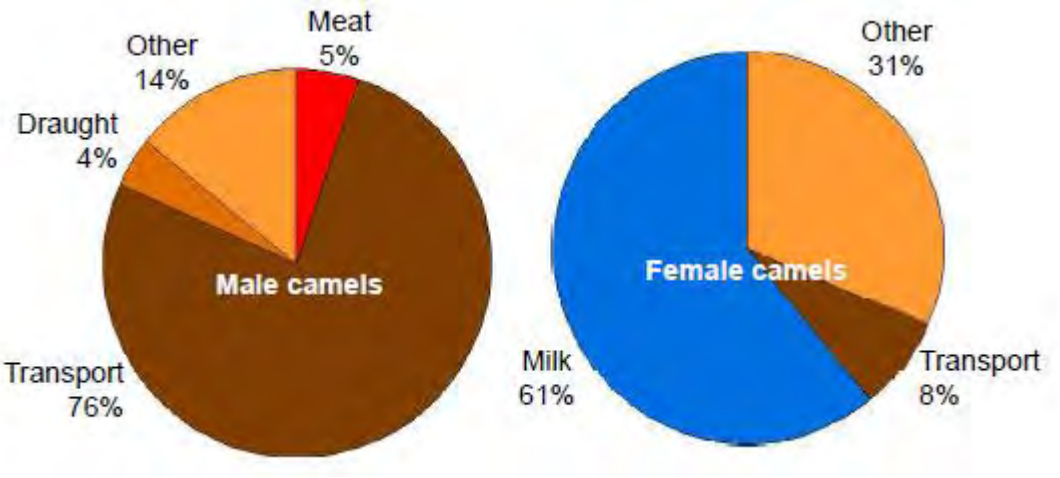


Figure 5: Uses of male and female camels in Ethiopia from CSA, 2013

The contribution of camels to the human welfare of developing countries is generally obscured by several factors, which tends to underestimate their true value. Consequently, less attention has been given to camel improvements for many years in the national development plans (Ahmad *et al*., 2010). However, camels are essential to supply animal protein to human in these marginal areas, contribute to the maintenance of rural activities and economical development, and finally facilitate the integration in the global economy (Faye *et al*., 2008).

1.3.1. Camel meat

The camel meat production is estimated to be very low as compared to other farm animals (FAO, 2006), however, information are quite difficult to collect since informal markets are the main part of the camel meat involvement. In Ethiopian situation, camels are slaughtered when respected person in the community dies, and during wedding or any religious or national or regional holidays. Traditionally, camel meat consumption is not common in a subsistence system, the size of the

carcass needing to share the meat between a wide numbers of people. However, the urbanization has increased the camel meat demand in most of the arid countries.

1.3.2. Camel milk

Their role as source of milk is particularly at times of drought, a common place phenomenon which drives most of all other species of animals out of milk. Camel milk is highly nutritious containing niacin, Vitamin C five times higher than cow's milk, ten times iron and very high calcium level. It also contains only 2% fat molecules joined to protein, so there is no stress on the liver to process fats. Camel milk is considered a complete food and can be consumed exclusively meeting all nutritional requirements for the pastoralists (Agrawal *et al*., 2005). Camel fat also contains higher concentrations of long-chained fatty acids (C14-C18) than short chained fatty acids, and is therefore healthier. Camel milk does not coagulate easily. It passes the acid stomach unchanged and reaches the intestines for absorption. Approximately 90%-95% of black individuals and only 20%-25% of white individuals throughout the world have partial or complete lactose intolerance. Although the lactose content in camel milk is as high as in cow milk, camel milk lactose intolerance does not occur. The reason is also not yet known (Wernery and Wernery, 2006). Camel milk is the sole source of protein in the desert where other sources are unthinkable (Figure 6).

Figure 6: Camel milk: Blessing of the desert for the pastoralist (Photo by Simenew Keskes 2012 in Afar region)

In general, camel milk is healthy and has the following advantages in comparison to cow milk. It can be produced in a non air-conditioned environment. It has five times higher Vitamin C content, rich in iron, contains 50 % less fat, reduces cholesterol and compatible among people who are lactose intolerant.

In Ethiopia there is a robust camel milk trade in the camel rearing regions, which is expanding and becoming lucrative. In Somali Region alone milk is marketed locally and to neighboring countries such as Somaliland and Djibouti on a daily basis (Figure 7). In Afar, it is becoming common to see pastoralists to sell camel milk for passengers along the road to Djibouti and Addis Ababa. The Borana pastoralists are also selling camel milk for nearby towns and Kenya Moyale.

Figure 7: Camel milk marketing in Somali region, Fafen district to transport to nearby towns (Photo by Simenew Keskes 2012 Somali region)

At global levels, about 85% of the milk produced and marketed throughout the world is cow milk which attributes to several factors including attitude to consume camel products due to religious and cultural issues, market accessibility, lack of investment in camel production, and so and so forth. However, the dairy potential of camel appeared higher than that of the cow reared under the same climatic and feeding conditions. In recurrent drought-stricken areas of Africa where severe drought frequently decimates cattle, sheep and goat populations, only the camel can survive continuing to produce milk (Muli *et al*., 2008). In Ethiopia, the Afar farmers rearing simultaneously cattle and camel get on average 1 to 1.5 litres of milk with afar zebu against 4 to 5 liters with Dankali camel (Schwartz, 1992; Faye *et al*., 2008). While

the greatest amount of camel milk in the world is consumed fresh or as naturally fermented product, camel milk has a wide range of products including: yoghurt, cheese, ice creams, puddings, chocolates of different flavors, butter, beauty products: anti-wrinkles creams; camel milk cleansing soap bars etc are some of the fermented camel milk products (Muli *et al*., 2008).

One of the remarkable features of dehydrated camels is their ability to continue lactation and to secret milk that is highly diluted with over 90% water content. A 600kg camel for example, has about 200 liters of fluid in the alimentary tract, which is available for milk production, giving 20 liters per day for 10 days (Yagil, 2000).

1.3.3. Medicinal perspectives of camel products

The medicinal properties of camel products were known to Arab physicians centuries ago. Camel milk, meat and urine have been used for medicinal purposes in many countries (Haddad, 2006). The composition of camel's milk is believed to be unique in terms of antioxidative factors such as vitamin C, lactoferrin, and components of health-related effects (insulin). However there is a need for more studies on characterization of the active components and apply into laboratory animals (Mohamed *et al*., 2005).

Camel milk has been used to cure diseases caused by chronic liver problems like jaundice, oedema, and swelling of the belly (Haddad, 2006). Recent research reveals that raw camel milk contains insulin-like proteins that can bypass the stomach and be absorbed intact (Agrawal *et al*., 2005). This characteristic of camel milk could help to treat diabetes develop pharmaceutical products. According to Haddad (2006), camel milk is used therapeutically against oedema, jaundice, problems of the spleen,

tuberculosis, asthma, anaemia, tuberculosis, and haemorrhoids (piles), and also used for improvement of bone formation in Indi and in Russia and Kazakhstan, doctors often prescribe camel milk to convalescing patients. Some positive effects of camel milk had already reported against sever food allergy in children with rapid and long lasting though it needs further researching (Shabo *et al*., 2005). According to Mehari *et al.* (2007) camel milk is used against gastritis, asthmatics, tuberculosis, urinary problems, hepatitis, jaundice, constipation and diabetics in Ethiopia. In addition to the above uses of camel milk, there are also reports about its cosmetic values. The secret of Cleopatra's beauty is bathing herself in camels' milk. Chocolates, chewing gums, ice-cream, shampoos and other products are availed in several supermarkets of USA, Europe and Asia.

Camel meat has been used since the late sixteenth century in traditional Chinese medicine. Camel meat is used to improve resistance to disease, to strengthen the muscles and bones, to moisten the skin, and to relieve internal pain. The fat extracted from the camel's hump is used to effectively relieve pain and swelling. Many Chinese restaurants serve, or plan to serve, their customers camel meat. Although camel meat is not commonly consumed in many parts of China, people are increasingly interested in tasting the meat (Liu, 2006). In Australia, the National Heart Foundation has endorsed camel meat for human consumption (Ellard and Seider, 2000).

In Arabic medicine camel urine is a standard prescription and still remains a staple of Bedouin natural remedies and endorsed by mainstream modern medicine. It is widely believed that camel urine has diuretic agents due to their browsing nature from wide varieties of desert plants having medicinal values (Haddad, 2006). Scientists are also dealing with camel tear and milk ingredients as curative agents for Alzheimer's diseases and positive signals have been reported (Louis, 2014).

1.*3.4. Other products and uses of camels*

Camel hide, wool and dung are also products used by human in different ways. The processing of camel hides is common in Northern Africa and the Middle East (MacNamara *et al*., 2003). Italy and the United States are the major market destinations for camel leather products. The good news from camel hide industries are the natural scarring effect does not diminish a hide's quality as it makes each one unique (Goulding *et al*., 2007). In Ethiopia, no body collects and process camel hide and the nation losses quite considerable income from this resource. In Kenya the price of one camel hide is equal to four oxen hides. This indicates that camel hide processing is a good venture to take part as a business for any interested body in East Africa where there are huge resources. Camel wool is harvested and processed for making padded cloths, quilts, and mattresses. In Asia and Latin American countries, quite large tones of wool are collected annually. Dromedary camels in some areas of North Africa and Asia produce wool though all breeds are not meant for wool production. In desert areas where fire wood is even unthinkable, camel dung is used to cook their food by nomads in most arid areas of Afro-Asiatic belt.

Transport of goods and human from place to place, camel racing in different Arab countries, camel wrestling in Turkey, camel jumping in Yemen, Sign of wealth/social values and status determinants in the pastoral society are also their other contributions to mankind. In Africa use of camels as renting animal to transport in tourist sites and countryside where other wheeled ways of transport across sandy desert is unthinkable are main means (Figure 8). This adds their value as means of significant income to the pastoralists (Aklilu and Catley, 2011).

Figure 8: Use of camels to transport goods from place to place where other means are not available: Case of Afar pastoralists crossing Awash River (Photo by Wesinew Adugna 2012 in Afar region)

1.3. 5. Ecological benefits of camel

The traditional nomadic husbandry of camels had only a slight negative influence on the desert ecosystem (Al-Rowaily 1999; Olsvig-Whitta ker *et al.* 2006). They eat anything that grows in the desert especially during starvation; however their primary preferences are thorny plants, dry grasses and saltbush. Per day they brows 8-12

hours and almost equal time are spent for ruminating (Oakland Zoo, 1993). Camels forage over large areas and eat only a few leaves from each plant. This has environmental benefits as they are very friendly with the desert plants and otter herbivores. This behavior contributes to combats desertification and optimally suited to areas with a low carrying capacity. The flat leathery pad-like feet are gentle on the sand soil surface and avoid exposing the soil for erosion during flooding as other sharp cloven hooves of ruminants. Camels also have a very efficient feed conversion rate and according to some calculations require only 1.9 kg of dry matter to produce 1 liter of milk, compared with 9.1 kg in cows (Oakland zoo, 1993). Camels are generally considered as environment friendly and are not associated with environmental degradation.

In general, the socioeconomic role played among the pastoral society emphasis on camel husbandry as a priority strategy to recurrent drought particularly in arid and semi arid areas of Africa. The trade of live camels to North Africa and the Middle East is also vibrant and prices are higher than at any time in the last 15 years, a great financial benefit for families with surplus camels to sell. In addition to all the above benefits of dromedary camels, in Ethiopia live camel trade value in 2010 was close to 61 million USD which is greater than the annual export values of other animals combined together (Aklilu and Catley, 2011).

1.4. Major Problems of Camel Production

Camel production is still facing several challenges to flourish as it should be. Among the major challenges emerging and re emerging disease, feed and water shortage, encroachment of vegetation with non edible bushes, calf mortality, abortion storm, recurrent drought, tribal conflicts, civil wars in the camel rearing areas, human settlement which limits free movement of the camels played significant hindrance to

the camel production sector. Land degradation in arid and semi-arid areas resulting from various factors, including climatic variations and human activities is increasing. Rangelands are severely degraded with most of its palatable perennial grasses and shrubs dramatically reduced. It is important to reverse the degradation processes through adopting a strategy for natural resource management systems. This strategy should be holistic and promote self-sufficiency of the desert people and provide means that ecosystems may be fully utilized on a sustainable basis.

In Ethiopia, studies have been conducted pertaining to the production constraint and reported long lists of disease as main bottle neck among the camel rearing pastoral society. The diseases include trypanosomiasis, camel pox, ecto-parasites and balantidiasis (Tefera & Gebreah, 2001). There are also other emerging and re emerging diseases including sudden death syndrome and Middle East Respiratory Syndrome which affect large number of camels in Ethiopia (Dawo, 2010; Chantal *et al*., 2014). Today, Livestock grazing affects more than 90 % of the land on the Arabian Peninsula and rangeland degradation takes place (Peacock *et al.* 2003; Gallacher & Hill, 2006). There is a shift from nomadic to sedentary farming led also to an increasing demand on natural fossil water resources because the number of camels and the fresh water irrigation of camel fodder plants increased with unchanged grazing areas of the natural vegetation in the pastoral areas of Ethiopia.

1.5. Future Prospects of Camel Production

Among the potential opportunities for future camel production; ever increasing population pressure, increased demand of animal products, awareness of the public, research output recommendations to policy makers and users as well as global climate change constitutes the main ones. Food consumption patterns towards livestock products are ever increasing. Over the last decade, consumption of meat in

the developing countries of Asia where the bulk of the world population increase has taken place. The animal products demand has been growing by about 3 percent per year, and dairy product consumption by almost 5 percent. Aggregate agricultural output is affected by these trends, not only through the increase in livestock production itself, but also through the linkage of livestock production to the crop sector which supplies feedstuffs, mainly cereals and oilseeds and the fisheries sector (FAO, 2013).

Globally, climate change represents a critical challenge to humanity in the 21stcentury. Developing countries are believed to be more vulnerable to the effects of climatic change due to their high reliance on natural resources, very limited capacity to adapt institutionally and financially, and high poverty levels (Thornton *et al*., 2010). The effects of climate change are more likely to be felt in extensive livestock production systems, and consequently, questions arise regarding options and strategies for adaptation by pastoral communities, whose livelihoods depend on climate-sensitive resources (Drucker *et al*., 2007). In Eastern Kenya camels were ranked as the foremost livestock, largely due to their high drought tolerant capability, while goat, sheep and cattle were ranked in decreasing order of magnitude of importance (Saidu and Omedo, 2010). Similar reports are also recorded in Ethiopia, It seems that prospects for camel milk are bright as it gives nomadic herders a rich source of income from the enormous demand for camel milk and milk products. The large demand for camel products are driven by the medicinal value on top of their daily food demand in each pastoralist family (Muli *et al*., 2008).

Global climatic change urges to use marginalized resources and camels are the future prospect. As climate change drastically alters the African landscape, raising camels could replace crops and other livestock in the hardest hot arid areas of the continent (Kaufmann and Binder, 2002; Jones and Thornton, 2008). The major impact of desertification are loss of biodiversity, and loss of productive capacity, such as the

transition from grassland dominated by perennial grasses to land dominated by non-palatable, toxic, thorny or halophyte shrubs. Therefore, climate change has both positive and negative impact in the future of camel production.

The potential of camels as economic income to pastoralists was dismissed; however, their value is now becoming increasingly acknowledged. It is expected to bring revolutionary changes and further improvements in enhanced milk production to put smile on the faces of the camel herders by improving their pastoral economy (Raziq *et al*., 2008). Increasing human population in the world has a risen the issue of food security. In order to combat with this issue, there is need to explore a new world of resources and camel can serve the best useful addition to the food supply chain in terms of milk, meat and other products (Ahmad *et al*., 2010; Abdi *et al*., 2011). Until very recently, camel research and public perception to their products were very minimal. However, it is becoming common to read reports related to camel from different perspectives though at its infancy stage.

CHAPTER TWO

The first part of the research section is based on the following three published articles and one unpublished study from Borana zone. (1) Simenew K., Mekuriaw M., Tesfaye S., Fekadu R., Wesinew A. and Fufa D. (2013). Reproductive Performance of Camels: Kept under Afar Pastoral Management System Using Progeny History Testing. *Journal of Camelid Science* 6:100-115. (2) Simenew K., Mohamed I., Berhan T., Tesfaye S., Fekadu R., Tesfu K. and Fufa D. (2013). Production systems and reproductive performances of *Camelus dromedarius* in Somali regional state, Ethiopia. *Journal of Agriculture and Environment for International Development* 107 (2): 243-266. (3) Simenew K., Dejen T., Tesfye S., Fekadu R., Tesfu K. and Fufa D. (2013). Characterization of Camel Production Systems in Afar Pastoralists, Northeast Ethiopia, *Asian Journal of Agricultural Sciences* 5(2): 16-24. (All are annexed at the end of this dissertation).

2. PRODUCTION SYSTEMS CHARACTERIZATION AND REPRODUCTIVE PERFORMANCE EVALUATION OF DROMEDARY CAMELS AMONG ETHIOPIAN PASTORALISTS

ABSTRACT

A cross-sectional questionnaire survey and focused group discussions were conducted to characterize production systems and reproductive performances of dromedary camels among Afar, Somali and Borana Zone of Oromia region, Ethiopia. A total of 299 households were included in the study during the period of October 2011 to March 2013. Husbands dominate in their roles for camel husbandry practices and almost all of them are illiterates. About 68.2%, 98% and 48% of Afar,

Somali and Borana pastoralists respectively preferred camels as their first choice over other livestock species kept mainly for milk and meat production as well as transportation. The camel management dominating in the study areas of Afar and Somali regions is traditional nomadic. Mature female camels were dominant and in the camel herd of Somali (54.87%) and Borana (33.8%), but no such numerical data could be found from Afar as they did not want to disclose their herd size. In Afar camels, mean± SD age at first calving was 64.7±8.9 months and their calving interval was 31.2±8.7 months. Mean± SD age at first calving and calving interval were 62.16±10.44 and 23.28±3.36 months respectively among Somali camels. Among Borana camels 69.6±7.2 months of age at first calving and 28.8 ±5.3 months of calving intervals were recorded. Age at first calving and calving interval could be minimized to a reasonable time by proper husbandry practices and health care. The mean lactation lengths were not statistically different among the three regions. Diseases and predators were reported as the main causes of calf mortality in the study areas. Calf mortality rate could be reduced by preventing predators attack and if other disease prevention and management cares were in place it could also be reduced to minimum. Animal diseases, lack of pasture and security were the main constraints in camel production of the study areas. For the better productivity of camels, the major constraints such as disease problems, lack of pasture and tribal conflicts should be mitigated. Proper husbandry and health services can play significant roles in the long term improvement of camel production and productivity of the region. Taking into account the adaptation of camels in the drought prone area and their productivity potential, policy makers, researchers and funding agencies should give attentions to camel health problems and improve food security in the ever widening desertification in the region.

Key Words: *Camelus dromedarius*, Ethiopia, production system, reproductive performances

2.1. Introduction

In situations where rainfall is scarce and unpredictable, pastoralism is a more appropriate livelihood strategy than rain-fed agriculture (Hatfield and Davies, 2006; Raziq *et al*., 2008; Gwida, 2010). The increasing human population pressure and declining per capita production of food in Africa precipitated an urgent need to develop previously marginalized resources and to optimize their utilization through appropriate livestock production systems (Wardeh, 2004; Mehari *et al*., 2007; Khanvilkar, *et al*., 2009). Camels contribute significantly to the livelihood of the pastoralists and agro-pastoralists living in the fragile environments (Abbas *et al.,* 2000; Tura *et al*., 2010). The majority of world's camel population is of dromedary type except small population of Bactrian camels in central Asia. World Camel population is estimated to be around 25.89 million spread across 47 countries; where Somalia has the highest population of 7 million followed by Sudan 4.25 million and Ethiopia 2.4 million (FAO, 2013).

Though dromedary camels are major component of the agro-pastoral systems in vast pastoral areas in Africa and Asia, little is known about its production potential and production systems compared to other domestic animals. Poor management practices in the regions where most camels are raised, adversely affect its reproduction and productive performance (Tibary *et al*., 2001, Kaufmann, 2005). Camel production could be a profitable venture for utilizing the vast arid and semiarid areas of Ethiopia where other animals thrive with difficulty, especially due to the recurring drought conditions. The reproductive performance of camel depends on the genetic potential of the species and breed, the management and the production conditions (Kaufmann, 2005). Dromedaries are believed to have reduced fertility compared to other domestic species due to many factors, but one of the most important is reproductive efficiency. Full exploitation of camels for milk and meat production would only be possible when their reproductive performance is properly understood and improved (Skidmore, 2005; Kalla *et al*., 2008; Hermas, 2009). The importance of maintenance

of high levels of reproduction in camel is not only for profitable production but also to provide ample opportunities for selection and genetic improvement. The reproductive efficiency of dromedary camels is very low (Abdel-Aziz and Nasser, 2013) and is a major problem in C*amelids* (Tibary *et al*., 2001). It remains a major obstacle to the growth of population of dromedaries (Babiker *et al*., 2011).

Information about camels in terms of productive and reproductive potentials in Ethiopia is very sparse. Only very few published research reports by Zeleke and Bekele (2000), Tefera and Gebreah (2001), Bekele *et al*. (2002), Biffa and Chaka (2002), Tezera and Kassa (2002), Mehari *et al*. (2007) and Megersa *et al*. (2008) were available and do not also represent the current situations. Therefore the objectives of this part of the research are to characterize camel production management systems and evaluate reproductive performances at their natural pastoralist management systems of Ethiopia.

2.2. Materials and Methods

2.2.1. Study area description

The study was conducted in Zone one of Afar, Jijiga Zone of the Somali and Borana Zone of Oromia Regional States (Figure 9).

2.2.1.1. Afar region

Afar makes the northeastern rangelands of Ethiopia with an estimated area of 96.7 thousand square kilometers and a total human population of 1.39 million. About 90.9% of the human population is rural inhabitants. The livestock population in the region includes 2.32 million cattle, 2.50 million sheep, 4.44 million goats, 859, 580 camels, 38,320 chickens, 187,450 asses, 3160 mules, 900 horses and 810 beehives. From these, the nomadic inhabitants possess 83.8.9% of the cattle, 90.6% of the sheep, 90.01% of the goat, 85.9% of the camel, 92.5% of the asses, 88.6% of the mule, and 100% of the horse populations in the region. The region has 5 administrative zones and 29 districts (CSA, 2005). In Afar National Regional State (ANRS) the study was conducted at selected camel rearing districts.

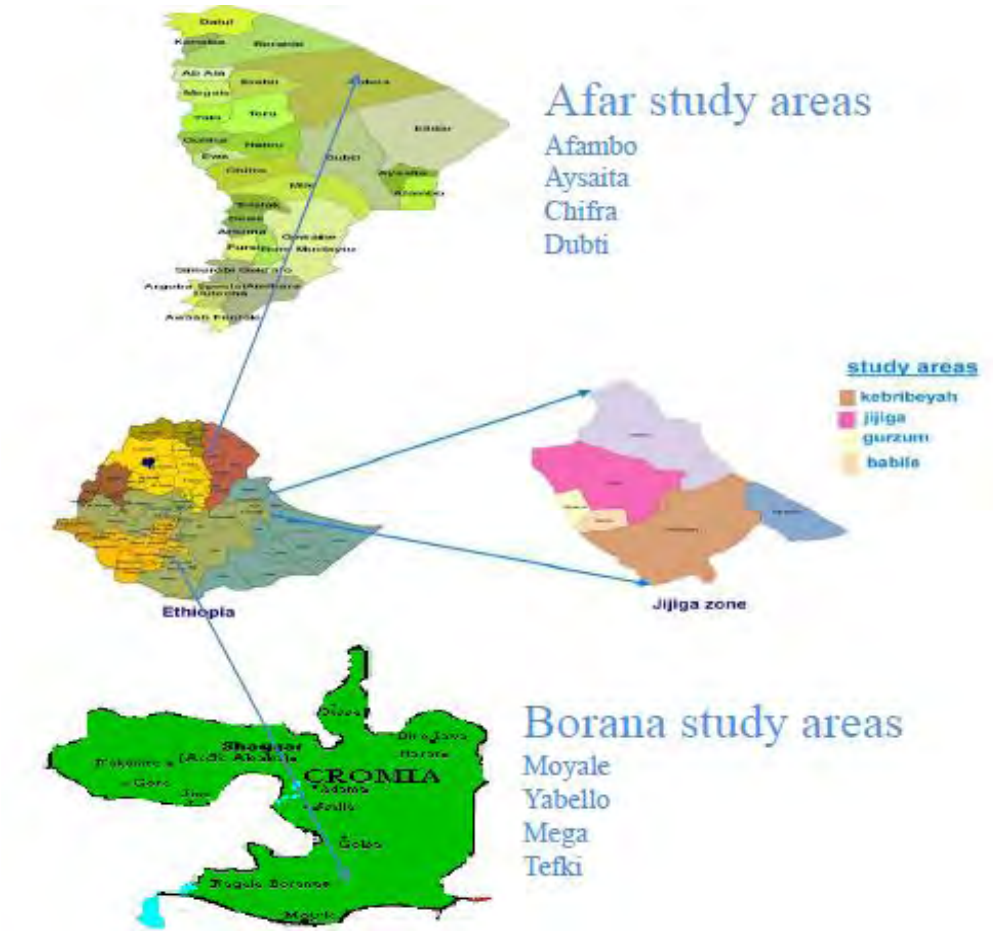


Figure 9: Map of study areas in Afar, Somalia and Borana, Ethiopia

Visits were made to the selected pastoral herds based on the pastoralists' willingness to participate in the study and to provide information. Much of Afar region is dry and rocky, unsuitable for cultivation. Out of the total area of the region, an estimated area of 97, 250 km^2, cultivated and arable land constitutes 5.24%, forest 1.54% bush and shrub 18.62%, grass land 1.56% marshy land 2.74%, water bodies 0.63%, and degraded and rocky land 63.7% Central Statistical Authority (CSA, 2011). The altitude of the area ranges from a minimum of 166 m below sea level to a maximum of 1500 m above sea level. Temperature varies from 25°C during the wet season to 48 ^{O}C during the dry season. Rainfall is erratic and scarce, and annual precipitation ranges from 200 to 600 mm. The region is frequently exposed to drought and is

classified as one of the drought prone regions in Ethiopia. The pastoralists use a lunar calendar in which each year is identifiable by its name (EEA, 2005; MoARD, 2008; CSA, 2011).

2.2.1.2. Somali region

Somali Region represents the largest part of the southeastern rangelands of Ethiopia with an estimated area of 279,252 square kilometers and human populations of 4.3 million. About 83% of the human populations are rural inhabitants. The region has 1.13 million cattle, 6.87 million sheep, 6.18 million goats, 1.21 million camels, 134, 190 asses, 154, 670 poultry, 160 mules, 50 horses and 5330 beehives. From this, the nomadic inhabitants possess 59.3% of the cattle, 93.3% of the sheep, and 89.5% of the goat, 86.3% of the camel, 31.8% of the ass and 100% of the mule and horse populations. The Region has 9 zones and 47 districts (CSA, 2005).

The Somali Regional State, which forms part of the Federal Democratic Republic of Ethiopia, is situated in the eastern part of the country at 4^0 to 11^0 North Latitude and 40^0 to 48^0 East Longitudes with a total land area estimated at around 325 thousand km^2 (MoARD-PADS, 2004). The topography of the Somali Region is mainly lowland, however, there are some spots that are relatively high. The altitude ranges between 500 and 1, 600 meters above sea level. The topography in Jijiga zone consists of complex features comprising of flat to gentle slope, hilly and mountainous. The average annual rainfall ranged from 300 mm to 500 mm and average monthly temperature ranges 16 to 20^0C (MoARD-PADS, 2004). Data collected from Jijiga for the period 1988 to 1998 indicate that areas with Jijiga terrain and its surroundings received annual rainfall that ranges from a minimum of 340 mm to a maximum of 627 mm, which averaged to about 500 mm per annum.

Jijiga has a bi-modal rainfall pattern, usually occurring during the months of March to May and July to October (IPS, 2002).

Jijiga administrative zone is divided into three separate Food Economy Zones (FEZs), namely, sedentary agriculturalists, agro pastoralists and pastoralists. Agro-pastoralism is a dominant production system in Jijiga Zone. Jijiga Zone comprises seven woredas namely Awbare, Dadamane, Gursum, Harshen, Jijiga, Kabribeyah and Tulli-Guled. Among these woredas, Dadamane, Gursum, Jijiga, Kabribeyah and Tulli-Guled were selected for conducting the study due to relative accessibility and camel population density purposively. The population in Jijiga Zone is mainly from Somali tribes which are Muslims and agro-pastoralists. Household size averages 6 and 5.3 heads in rural and urban areas, respectively. The zonal household size averages to 5.9, which is less than the average of 6.7 for the Somali Region (JZOA, 2001). This area represents the heartland of camel population of the country.

2.2.1.3. Oromia region

Oromia has an estimated area of 353,006.81 square kilometers and human population of 26.6 million among which about 86.7% are rural inhabitants. The region has 17.21 million cattle, 6.91 million sheep, 4.85 million goats, 139, 830 camels, 959,710 horses, 63,460 mules, 278,440 asses, 11.64 million poultry and 2.51 million beehives. The region has 12 zones and 180 districts. Borana Zone and Fentale Districts are the areas where camel is reared (CSA, 2005). The climate of the Zone of Borana is largely semi-arid with relatively cool annual temperatures (19-24^{0}C) and a mean annual rainfall ranging from 400-600 mm that is bi modally distributed (60% during April to May and 30% during October to November). A cool dry season occurs from June to September, while a warm dry season occurs from December to March vegetation is comprised of mixed perennial savanna characteristic of East Africa.

The Borana zone is one of 13 administrative zones within Ethiopia's Oromia regional state. It is located in the Southern part of the state (between 3°36 - 6°38'North latitude and 3°43'- 39°30' East longitude) and borders Kenya. Yabello is the capital town of the Borana zone and lies 570 km south of Addis Ababa. The zone covers 48,360 km^2 of which 75% consists of lowland; the zone frequently is exposed to droughts. The zone consists of eight districts covering 275 "Gendas" (the lowest administrative unit). There are 19 urban centers, of which 10 have town administration. The zone is inhabited by almost one million people (CSA, 2008). The Borana rangelands cover about 1.9 million hectare of land. The region has a semi-arid savannah landscape, marked by gently sloping lowlands and flood plains vegetated predominantly with grass and bush land. The geology is composed of a crystalline basement with overlying sedimentary and volcanic deposits. People are predominantly involved in small-scale subsistence agriculture production and mainly on livestock husbandry. There are no perennial rivers and rainfall varies highly, both spatially and temporally.

2.2.2. Selection of study areas and study population

A preliminary survey of camel breeds/types, their historical origin and distribution were carried out by organizing a consultative workshop in which tribal elders, camel herders, extension workers and livestock production experts in the three regions and investigators and co-investigators from host and partner institutions are involved. Attempts were made to involve representatives from each zone where camels were currently kept by pastoralist or agro-pastoralists. Once, the camel breeds/types were identified, the specific study sites (zones and districts) and study populations were selected purposely for data collection taking into account the availability of the identified breeds in the zones and districts.

2.2.3. Study animals

Camels were the study animals considered in this research and information about the production system and reproductive parameters determination in the study sites. In Afar, 293 female camels and 792 parturitions were considered. In Somali region, the study animals considered for this study were 364 female camels and information about the reproductive parameters was collected. In Borana, 182 female camels and 341 parturitions were included in this study.

Physiological status (dry, lactating or pregnant) and previous breeding history of the herd were obtained by using the progeny history questionnaire (Kaufmann, 2005). This method relies on the herders' intimate knowledge of their camels and long lasting memory of their life history. Age of the camels was determined by examination of eruption of the permanent incisors as described by Dioli (2013) and also most importantly by questioning of the herd owners. In almost all the cases our age determination coincides with the information from the owners. In cases of differences between the two techniques, we go for dental information as the owners sometimes might forget the ages of some camels.

2.2.4. Study design and data collection methodology

For the production system characterization in the study areas, 110, 100 and 89 camel owners from Afar, Somali and Borana respectively were participated in individual questionnaires and focused group discussions. A cross sectional study design and set of detailed structured questionnaire were used to collect information from camel owners in different sites by guided interviews. Focused group discussions were the other approach to gather information about the production system and reproductive

performances of camels among pastoralists. The questionnaire was previously used in the Sudan and Saudi Arabia and found to be effective for such studies (Algaylia and Mansour, 1998; Ishag and Ahmed, 2011) and adopted for the current research with some modification to fit in the Somali situation. Observational studies were also applied in the study areas in addition to historical information about the pastoral system and herd production collected from direct questioning of the pastoralists. Primary and secondary data were the sources of information used for the study. Primary data sources were the key informants during guided interviews and group discussion in the respective selected districts and the secondary data were collected from different regional, zonal and district level agriculture and pastoralist offices.

Purposive sampling procedure was implemented because of difficulty to apply random sampling due to the mobile, scattered and less accessible nature of pastoral communities. The household heads were selected based on camel possessions and willingness to be part of the survey. For group discussion, 15 camel breeders with long experience in camel rearing were selected from the total households and interviewed to gather reliable information on the production system and reproductive performance variables. The questionnaires were designed to obtain information on general household characteristics, livestock and herd structure, herd management, breeding practices, disease prevalence, production objectives, feeding management and production constraints. Also information on the general composition of the herd, breed, age, sex, purpose of keeping camels, reproduction and production parameters were collected for this study.

2.2.5. Data analysis

The collected data were coded and entered in to Excel 2007 spread sheet for storage. The SPSS statistical computer software version 20 was used to estimate the means

and standard deviation or standard error of the mean for the reproductive parameters considered. Frequency distribution figures were also calculated for relevant reproductive and production parameters. Age at first calving was calculated as the time span between date of birth and date of first parturition for breeding females for both female camels born in the herd and these heifers acquired from known herds. Calving interval was calculated as the time span between two subsequent parturitions. Abortion rate was calculated as the number of abortions per number of all pregnancies recorded based on the owners' information. Calf mortality was calculated as the number of calves born alive but died before weaning (at 12 months of age) per number of all calves born. Results were presented mainly in the form of narrations, tabular summaries and figures.

2.3. Results

Somali pastoralists who have indigenous knowledge on camel reproductive performance and production system were interviewed. Major constraints on camel production, farming system and important reproductive performance parameters were studied during the research.

2.3.1. Demographic characteristics

Among the Afar pastoralists participated in the study, none of them had gone to school. In Somali pastoral areas of the study sites education service was also very poor and most of the respondents in the study areas were illiterate (94%) whereas the remaining (6%) got only primary education of elementary schools (Grades 1-7). However, among the 89 Borana pastoralists who have participated in this study, about 17% of them went up to elementary schools and 3 of them up to high school.

All the respondents were males since camels are only owned by males in all the study areas. The age of household head varied between 15 and 97 with mean age of 38.6±13.5 years.

2.3.2. Management system

Husband occupied the highest sharing in camel management followed by sons and hired laborers (Table1). Wives took part in camel management with husband and sons in milking, feeding, housing, herding and marketing in Afar and Borana pastoralists. Women are not allowed to herd camels since it is a difficult work and camels browse until night time which will cause a risk if women keep camels among Somali pastoralists. Hired laborers have increased in their numbers for camel herding in Somali pastoral areas.

Feeding practices in the Afar camel production system in the study area is browsing 43.6%, and browsing and mineral supplementation 56.4%. In the present study, Afar pastoralists migrated mostly in the dry season to search for pasture, water and to prevent their animals from diseases occurring during those dry seasons in the area. Very rare migration occurred during rainy season to high land areas due to the Awash River flooding problem. Based on the season of the year, age, physiological and health status of the camel, supplementary feedings mostly mineral supplement is practiced by the Afar pastoralists. But during dry season, the camels are supplemented with ever green plants when calves 4-5 months age to adapt grazing and browsing. Supplementation of minerals (salt) is done in 2-3 months intervals. Similarly in Somali and Borana pastoral areas, supplementation of browsing is rare and only mineral supplement was common practice in all study areas.

Table 1: Labor distribution in camel management among family members of pastoralists in Ethiopia

Family members	Management Practices						
	Feeding	**Milking**	**Breeding**	**Herding**	**Health care**	**Housing**	**Marketing**
	Afar (N=110)						
Husband	15.5%	26.4%	20%	10.9%	17.3%	14.5%	100%
Wife	10%	16.4%	14.5%	48.2%	20%	6.4%	0%
Son	0%	0%	0%	0%	0%	0%	0%
Husbands and sons	74.5%	57.2%	65.5%	40.9%	62.7	68.2%	0%
Husbands and wives	0%	0%	0%	0%	0%	3.6%	0%
Sons and wives	0%	0%	0%	0%	0%	3.6%	0%
	Somali (N=100)						
Husband	30%	26%	83%	50%	92%	70%	84%
Wife	0%	1%	0%	1%	0%	0%	0%
Son	16%	7%	3%	12%	3%	5%	1%
Daughter	0%	0%	0%	1%	0%	0%	0%
Hired laborers	2%	0%	0%	10%	0%	0%	0%
	Borana (N=89)						
Husband	32.5%	42%	91.2%	34%	92%	18.6%	96.3%
Wife	8%	12.8%	6.4%	6.8%	4.3%	5%	0%
Son	28.9%	8%	14%	20.6%	9%	10.1%	3%
Daughter	3%	0%	0%	6.2%	0%	4%	0%
Hired laborers	10%	3%	0%	21%	0%	7%	0%

N=number of household respondents included in the study

2.3.2.1. Type and number of livestock reared by Ethiopian pastoralists

During the study it was found that camels, cattle, goats and sheep were the types of livestock reared by pastoralists in the study districts (Table 2).

Table 2: Composition of livestock and herd size in Somali and Borana pastoralists of Ethiopia

Animal species	**Livestock herd composition**	
	Somali (N=100)	**Borana (N=89)**
	Mean±S.E.M	**Mean±S.E.M**
Camel	27.54±15.96a	18.2±6.4b
Cattle	13.49±11.54a	32.5±8.3b
Goat	7.87±12.62a	12.6±3.8b
Sheep	4.41±10.58a	16.3±10.4b

* Different letters of the same row indicated significant differences of herd size for each livestock species at $p<0.05$

Responses to the questionnaires related to herd size in Afar society was not possible due to traditional, misunderstanding and misinterpretations of such inquiries.

2.3.2.2. Relative importance of livestock among Ethiopian pastoralists

Camel, cattle, goat and sheep are livestock species in the ranking order of their relative preference as shown in Table 3. The majority of the respondents preferred camels as first rank to other livestock species followed by cattle, goat and sheep.

Table 3: Ranking of livestock among Ethiopian pastoralists based on their relative importance

Animal species	Rankings											
	Afar				Somali				Borana			
	First	Second	Third	Fourth	First	Second	Third	Fourth	First	Second	Third	Fourth
Camel	68	9	11	12	98	1	1	0	48	50	2	0
Goat	27	66	6	19	1	11	24	10	0	2	52	46
Cattle	4	4	43	19	0	66	8	8	22	45	3	0
Sheep	1	21	24	29	0	8	11	10	0	3	43	54

The numbers of respondents vary as some of them did not provide complete information and exclude from this specific part of the study.

2.3.2.3. Camel herd composition

Camel herd composition in the study areas are presented with their respective proportions in Figure 10.

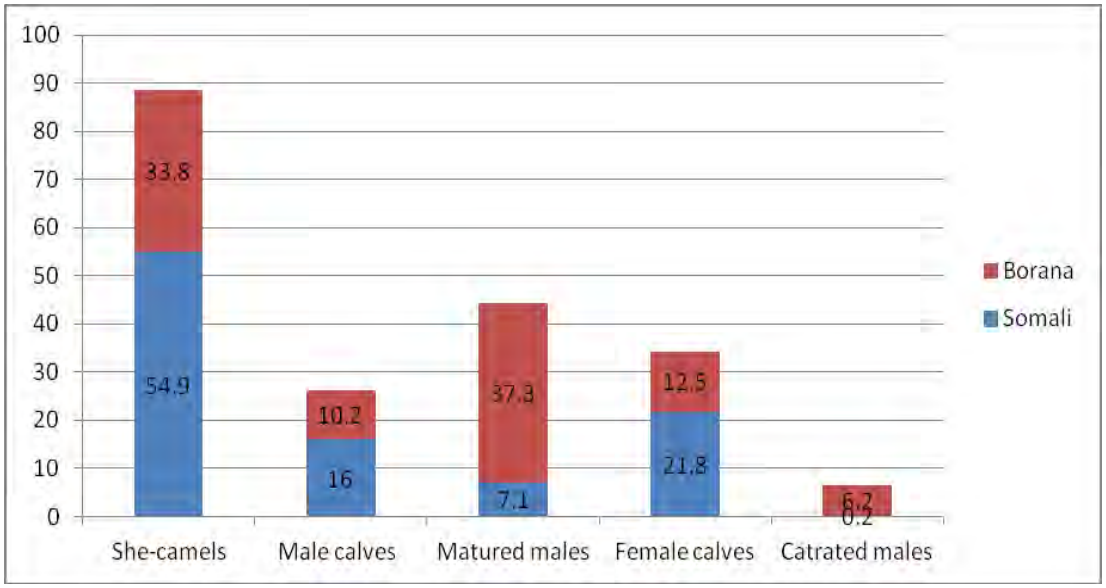


Figure 10: Camel herd composition in Somali and Borana pastoralists of Eastern Ethiopia

2.3.2.4. Camel management system and migration trends of households with their camel herd

Afar pastoralists covered long distance to neighboring Amhara and Tigray regions during the dry season during drought in search of food and water for their animals. Camels were primary stock and status indicators and represented the pastoral capital wealth of the Afar society and were essentially raised and kept for this reason. In Somali, traditional nomadic and transhumance management system accounted for 95% and only 5% of them were sedentary pastoralists in the study area. Camels were taken to mountain areas during wet season and to valleys, where pasture is available during dry season. Camels in the study area moved to different places in different seasons. During wet season, camels were taken to mountain areas (91%) where there is no tick infestation and flies to prevent tick borne diseases and foot wounds whereas (9%) of the pastoralists took their camels where ever there was a good

pasture. During dry season pastoralists move their camels to valleys (93%) where they could get cactus (*Opontiaficus-indica)* and water for their camels and the remaining 7% took where ever pasture is available. In Borana area, it was more of sedentary and mixed crop-livestock production where about 85.8 were growing crops.

2.3.2.5. Measures and reasons for culling female camels among pastoralists

In very rare cases old, infertile and sick female camels were sold and slaughtered otherwise they were kept in the herd until they die. The major reasons to cull female camels in the Somali society included; diseases (30%), old age (41.4%) and poor production performances (28.6%) with 99% of culling measures accounted for selling. The respective culling reasons and measures taken are presented in figures 11 and 12.

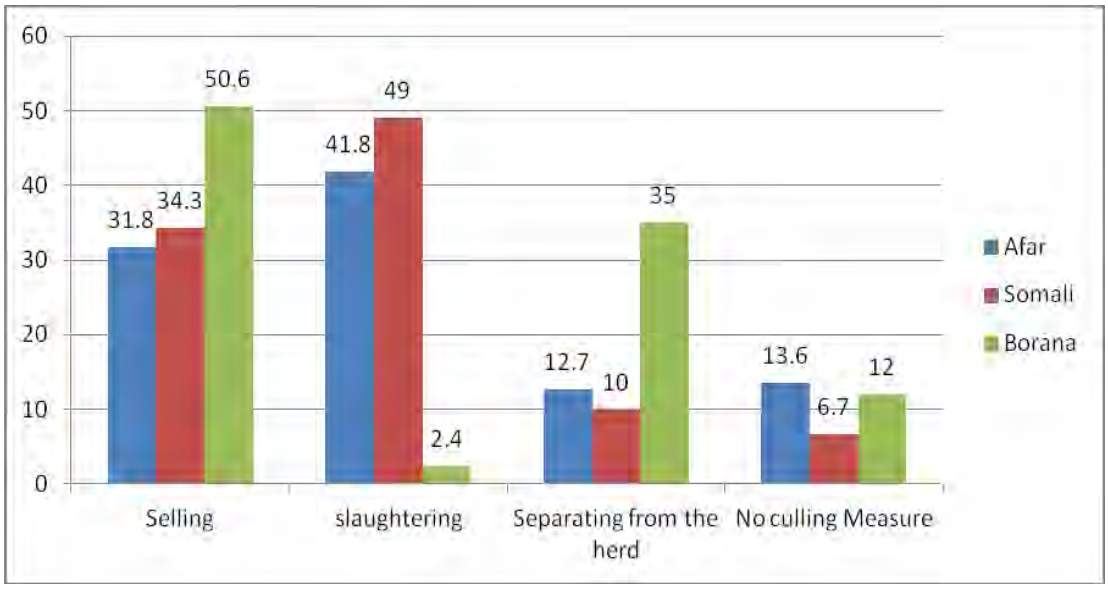


Figure 11: Culling measures taken by Afar, Somali and Borana pastoralists, Ethiopia

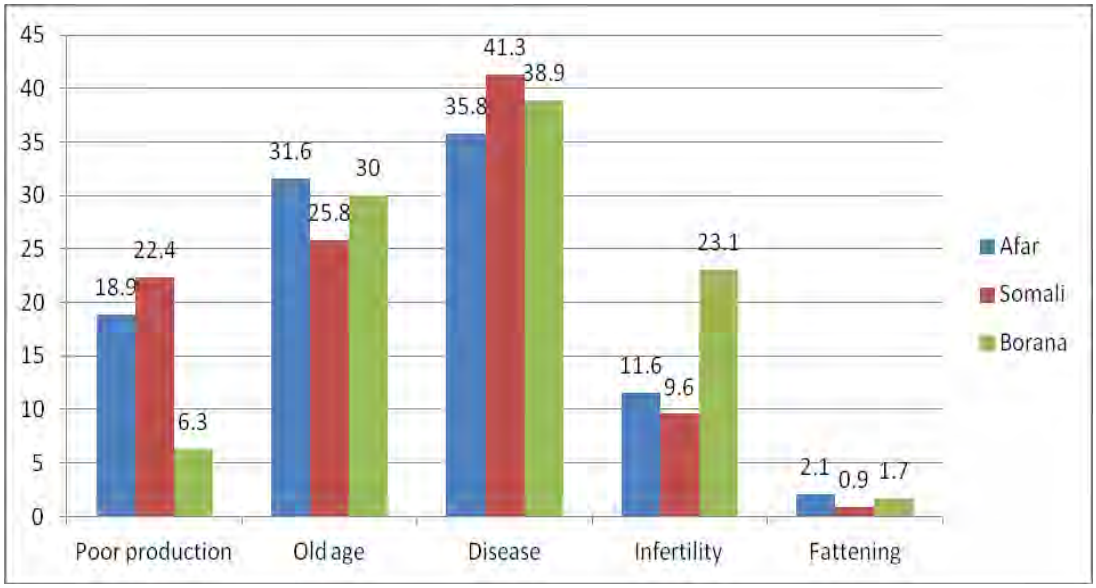


Figure 12: Culling reasons of camels among Afar, Somali and Borana pastoralists, Ethiopia

2.3.2.6. Camel herd dynamics simulation

Herd dynamics simulation data were developed in order to estimate long-term herd development with the status quo parameters and with improved reproductive parameters for the Somali camel populations. Some hypothetical improvements were tested for age at first calving, calving interval and calf mortality rate. Improvement of age at first calving and calving interval is conducted by excluding ages at first calving older than majority of the animals fallen and calving intervals longer than the data set resulted in a mean age at first calving and mean calving interval given in table 4 in the improved state.

Table 4: Mean age at first calving, calving interval and calf mortality rate of Afar, Somali and Borana camels in Ethiopia

		Reproductive parameters	Mean±SD (months)	Percentage (%)
Afar (N=293)	**Status quo**	AFC	64.7±8.9a	
		CI	31.2±8.7a	
		Calf mortality rate		12.4c
	Improved state	AFC(> 72 months of age excluded)	63.2±6.8b	
		CI (>36 months excluded)	29±5.9b	
		Calf mortality rate		7.7c
Somali (N=364)	**Status quo**	AFC	62.2±10.4a	
		CI	23.3±3.4a	
		Calf mortality rate		10.7d
	Improved state	AFC (> 60 months of age excluded)	57±5.5b	
		CI (>18 months excluded)	14.8±4.8b	
		Calf mortality rate		7d
Borana (N=182)	**Status quo**	AFC	69.6±7.2a	
		CI	28.8 ±5.3a	
		Calf mortality rate		18.6e
	Improved state	AFC (> 60 months of age excluded)	58.6±6.2b	
		CI (>18 months excluded)	19.8±5.4b	
		Calf mortality rate		12.8e

SD=Standard Deviation, different letters for the same reproductive parameters of each region to status qua and improved state signifies statistically different at $P<0.05$.

2.3.3. Breeding practices and reproductive performances of dromedary camel bulls

About 59% of camel herders kept only one breeding male and very few herders (8%) kept up to three breeding males. The sources of breeding camel in pastoralist production systems were own herd with 82% in Afar, 69% in Somali and 38% in Borana.

2.3.4. Production and reproductive performances of performances female camels

Daily milk yield of camels per day depends on feed availability, season and water access. Abortion rate among the camel herd in the study regions ranged from 8%-23%.

Table 5: Productive and reproductive performance of female camels under their pastoral production system Ethiopia

Production and reproduction Traits	Afar		Somali		Borana	
	N	Mean±SD	N	Mean±SD	N	Mean±SD
AFS (months)	293	47.6±7.3a	100	52.6±7.6b	182	55.2±9.1c
AFC (months)	293	64.3±8.9a	100	62.2±10b	182	69.6±7.2c
CI(months)	293	31.2±1.4a	100	23.3±3.4b	182	28.8 ±5.3c
GL (months)	293	12.1±0.2a	100	12.4±0.5b	182	12.5±0.8b
Longevity (years)	293	21.7±3.85a	100	18.5±5.5b	182	16.4±3.1c
Number of calves	293	8±3a	100	9.2±2.7b	182	6.1±1.2c
Number of services	293	1.6±0.9a	171	1.8±1.3a	182	2.6+1.2c
Milk yield at the start of lactation (L)	293	7.6±2.5a	364	4.4±2.0b	182	7.3±1.6a
Milk yield at the middle of lactation (L)	293	2.5±1.9a	364	3.8±2.3b	182	3.8±1.3b
Milk yield at the end of lactation (L)	293	3±1.3a	364	1.7±0.9b	182	2.1±0.9c
Lactation length (months)	293	12±3a	364	11.5±1.9b	182	12.8±3.1c

N=total number of camels included as part of the study for the respective parameters listed and SD=Standard Deviation, AFS= Age at First Service, CI= Calving Interval,

AFC= Age at First Calving, GL=Gestation Length. *Same letters across same row represents no significant difference among the three regions of same parameters considered in that particular row.

2.3.4.1. Camel production objectives among Ethiopian pastoralists

Milk and meat production were the main objectives of keeping camels in Somali pastoralists (100%). Animal sale, traction, insurance and investment were also mentioned to be objectives of keeping camel by the pastoralists.

2.3.4.2. Main constraints in Ethiopian pastoralist camel production

The main constraints of Somali camel production mentioned in the study area were diseases, lack of pasture, security and lack of capital (Figure 13).

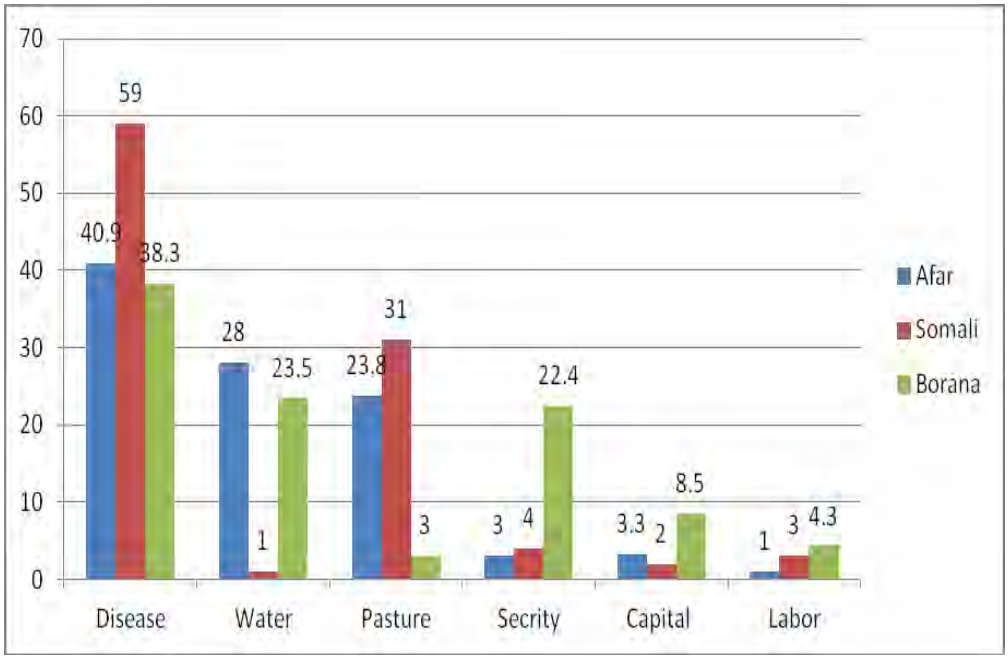


Figure 13: Production constraints of camel rearing in Afar, Somali and Borana pastoral areas of Ethiopia

2.3.4.3. Diseases of camels affecting production and reproductive performances

Diseases were picked as the major hindrance to camel reproductive performances in the study area. The major diseases that affect camel reproductive performances according to their relative importance were reported by the respondents as indicated on figure 14.

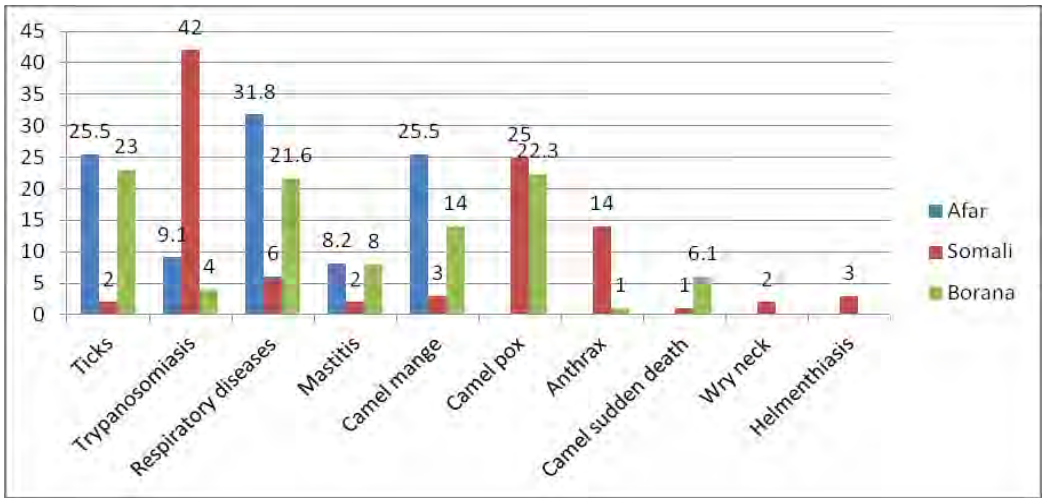


Figure 14: Diseases affecting performances of camels in Afar, Somali and Borana, Ethiopia

Trypanosomiasis took largest score in Afar followed by camel pox. However, in Somali respiratory diseases took the larger share and ectoparasite infestation was major problem in Borana areas.

2.4. Discussion

Camels are important livestock species among the pastoralist of arid zones of Ethiopia. Almost all of the pastoralists possessed mixed species of livestock including camels, cattle, sheep and goats. In the demographic demonstration, none of the interviewed Afar pastoralists went to schools. Only 6% of the interviewed camel owners went to elementary schools among Somali pastoralist and it seemed that there were better opportunities for Borana pastoralist to get access to school as a number of them went even to high school levels. When we compare the educational status of pastoralists of this study with Ishag (2009) report in Sudan was lower and the Sudanese pastoralist went to college and graduated though few in number. All of the respondents were males and were in adult age groups. It seems that in the pastoralist system women and youth involvement in camel ownership is very rare. Husband occupied the highest share in camel management followed by sons and hired laborers (Table1). From the three camel rearing regions, Borana pastoralists allowed relatively fair involvement of family members for camel management.

The findings of the present study in Somali and Borana on livestock composition and herd size were comparable with the studies of Mehari (2006), Eyassu (2009) and Ishag (2009). In Afar, it was impossible to get information about their herd size due to their tradition beliefs that telling the number is not good for their herd size. It was indicated that keeping different livestock species by pastoralists is beneficial to sustain the pastoral livelihood during the worsening impacts of drought (Desta and Coppock, 2002). Camel was ranked first over all other livestock species in all the three regions. Camel herd composition in the study areas showed that she camels and female calves dominated the camel herd composition and this was in agreement with that of Darosa and Agab (2008), Megersa *et al.* (2008) and Al-Dahash and Sassi (2009).

Larger proportions of females in herds in the area indicate a strong desire of herdsmen to maximize herd size and the importance of camel milk production in pastoral areas. She camels were more dominant in the herd to ensure effective breeding and food security through market exchange of livestock (especially small ruminants) (Yayneshet and Kelemework, 2004). Borana pastoralists kept more number of castrate males as pack animals and for marketing purposes. Slow rates of reproduction and long gestation intervals were also considered as factors that result in higher proportions of breeding females (Wilson, 1998). Pastoralists had indigenous knowledge to select the superior dams that give more female calves than males. The mean camel herd composition of pastoralists obtained in this study was comparable with that reported by Ishag (2009) in Sudan.

The majority of the respondents preferred camels as first rank to other livestock species followed by cattle, goat and sheep. Present study holds agreement with previous studies of Adugna and Aster (2004), in Dirre and Moyale districts of Borana zone in Oromia Regional State and in Moyale district of Liben zone in Somali Regional State in southern Ethiopia. In Somali pastoral area as is true in most of other pastoral systems camel was the best adapted animal because of its ability to resist drought and thirst by their special physiological and anatomical adaptation mechanisms to other domestic livestock species. Somali societies believed that camels were holy animals and can't be compared to other livestock. Camel is the only animal used to determine compensation for homicide, a lost eye, teeth, broken bones, and so on, depending on the circumstance and social status of the victim and the aggressor (Adugna and Aster, 2004). According to respondents, the reasons they preferred camels to other livestock species were; tolerant to drought and to feed and water shortage, produce high amount of milk and meat, can be used as pack animal and can travel long distances.

Camels in the study areas moved to different places in different seasons. Afar pastoralists had to cover long distance to neighboring Amhara and Tigray regions, especially during the dry season during drought. Having to cover long distances with cattle in northern Afar always bears the risk that part of the herd perishes due to water or grazing shortage. But camels were primary stock and status indicators and represent the pastoral capital wealth of the Afar society and were essentially raised and kept for this reason. On the other hand, sheep and goats were considered consumer and market goods, which were frequently sold and traded for grains and basic household goods of primary necessity (ANRS, 2010). All the above mentioned reasons and facts lead the Afar pastoralists opt for camel rearing as their primary livelihood and it seemed that in the ever widening desertification and climatic changes, highlanders also start to keep camels with other species in the districts of Amhara and Tigray regions adjacent to Afar.

During wet season, camels were taken to mountain areas, where there were no tick infestation and flies to prevent tick borne diseases and foot wounds whereas pastoralists took their camels wherever there was good pasture. During dry season pastoralists moved their camels to valleys where they could get cactus (*Opontiaficus-indica)* and water for their camels and the remaining took where ever pasture was available. This finding was in agreement with that of Elmi (1989), Mehari (2006). Tezera (1998) reported that the households of Abasgul and Bertire clans in Jijiga moved between Kebribeyah through Jijiga highlands of Fafen areas in Somali pastoral areas. According to the respondents, the main reasons for migration were for search of forage, water, and salt. Pastoralists complained that nowadays there is no migration to far distance to get enough feed for their camels due to security and restriction of livestock movement to the neighboring regions. The study zones from the three regions were agro-pastoralist practicing crop-livestock farming system and others kept only livestock. This finding is comparable with the study of Ishag (2009) who stated that camel herders in Gezira (Sudan) practice crop-livestock farming system (61.5%) and 38.5% only livestock rearing.

Considerable animal exchange rates were exercised among Ethiopian pastoralists in the study areas. Number of deaths due to diseases and predator attacks were higher than those sold. This is in agreement with the study of Megersa *et al.* (2008). In Borana pastoralist the herd dynamics rate due to trade was much higher followed by Somali and in Afar pastoral areas less dynamism was observed. In Somali predators attack was common as herders reported and hyenas and lions prey their camels, but hyenas were the most dominant. In very rare cases, female camels were sold and slaughtered otherwise they were kept in the herd until they die in Afar and Somali, where as Borana pastoralist sold them for slaughter houses and to other needy population in the highlands of Bale area. The major reasons to cull female camels in the pastoral society were diseases, old age and poor production performances. Selling was the most common measure to cull female camels in Borana and Somali society but in Afar slaughtering was more common than selling.

Majority of camel owners used their own herds for replacement of the breeding male. This finding agrees with that of Ishag (2009) in Sudan. Camel owners who did not keep breeding male camels reported the small size of herd and death of breeding camel as the main reasons for absence of a breeding male camel. They were using bulls of their relatives, friends or mixed their herds with another herds with a good breeding male for free. According to the respondents most of the pastoralists kept only one breeding male camel in their herd and very few with three or four breeding male camels and others did not have any. This agrees with the finding of Melaku and Gebreah (2001). The reasons that pastoralists kept only one bull were to avoid fighting among bulls and to get similar types of offspring with good performance. The results also revealed that the average number of breeding male camels ranged from 1.15 in Afar to 1:50 in Somali herds. This finding agrees with previous studies of Mehari (2006) and Ishag (2009). During rutting period, the breeding male camel will not allow other mature bull to be in the herd and acts as king of the herd by showing signs like gurgling sound, urine splashing, splayed stance, frothing of saliva and poll gland secretion. The average age to select breeding male in Somali was

higher than in Afar. This may be due to management system difference, the climate condition of the area and also camel breed differences between the two regions. Somali pastoralists select their breeding male camel by genetic and appearance. The selected breeding male camel was not used for any purpose other than breeding. The average age of keeping breeding male camels in the herd in the study area varied across the three regions and was in agreement with the reports of Ishag (2009).

In the herd dynamics simulation analyses, the exclusion age margins were not arbitrarily chosen, rather majority of the camels gave birth every 18 months and their first birth at the age of 60 months from the data collected. Therefore, it is reasonable to set these months as age of possible improvement for the whole camel population in the study areas. This actually led us to choose longer than the mode values and this was also used by Kaufmann (2005).Proper housing to prevent predation by itself improves calf mortality and if other management measures like vaccination and good colostrums supplement might reduce calf mortality even better.

The simulated effect of reproductive parameters improvement on herd demography is very important aspect of progeny history testing techniques (Elbashir *et al*., 2012). If we can make attempts to counteract this development, the simulation results show that measures to reduce mean age at first calving, calving interval and reduction of mortality rates will be effective (Sahani *et al*., 2003). The comparisons of Afar camels with the Kenyan three camel breeds (Rendille, Gabra and Somali) show us both similarities and differences among them depending on the different reproductive parameters. Feed or water was found to be a problem during dry season (January-April). Camel pox, trypanosomiasis, calf scours and respiratory diseases were the most common diseases. Major camel reproductive problems reported by the groups were abortion, still birth, uterine prolapse, retained fetal membrane, dystocia and endometritis. All the pastoralists involved in the focused group discussion had planned to improve camel milk production through selection and proper management

including feed improvements. They had also informed about lack of public veterinary clinics among the pastoral community and requested to equip the existed ones for better services.

As far as raw milk and meat eating habit is concerned reported in different studies that there is unfortunately higher prevalence rate of zoonotic diseases such as brucellosis in their herds (Wesinew *et al*., 2013). The Afar people got the bulk of their food from milk; meat and butter in the past good days, and very recently have made a shift towards grain as a major component of their diet. This is partly attributed to (i) insufficient milk yield and loss of livestock due to above mentioned factors, (ii) their gradual integration into market whereby they exchange animal and animal products for grain and, (iii) exposure to relief food provided in the form of grain during the past famine crises and the resultants changes in food habits. In Somali and Borana areas, boiling of milk was also done though most pastoralists used fresh milk to drink. Camel milk marketing had been just stared in Afar along the road sides to Aysaita and Samara, however, in Somali and Borana it has been practiced here and there though no well established association to milk marketing was observed.

Generally there was a total lack of a recording system in all studied areas. None of the interviewees reported that they recorded the performance or health status of their herds and this should be changed for improvement of their herd performances. The good news is every member of the family in the pastoralists knows about each and every camel life history and this was typical in pastoral community.

CHAPTER THREE

This section of the dissertation is based on manuscript submitted for publication: Simenew K, Moa M., Ashenafi F., Tilaye D., Fekadu R., Getnet M., Gizat A., Tesfaye S. T., Tesfu K. and Fufa D. An abattoir survey of lesions of the internal reproductive organs of female dromedary camels in Ethiopia (*Submitted to BMC Veterinary Research)*.

3. AN ABATTOIR SURVEY OF LESIONS OF THE INTERNAL REPRODUCTIVE ORGANS OF FEMALE DROMEDARY CAMELS IN ETHIOPIA

ABSTRACT

A cross-sectional study design was devised to characterize the pathological lesions and to isolate possible bacterial causes of reproductive failures in *Camelus dromedarius* slaughtered at Akaki abattoir. Reproductive organs of 231 female dromedary camels (169 from Borana and 62 from Afar) were grossly inspected and samples were collected for histopathological and bacteriological studies. A total of 46 (19.9%) camels were found with reproductive organ disorders of follicular cyst (3.5%), ovarian hypoplasia (0.43%), ovarian tumor (0.87%), para-ovarian cyst (0.87%), infundibular cyst (0.43%), uterine serosal cyst (0.43%), endometritis (13.4%) and endometrial calcification (0.43%). Uterine abnormalities were the major disorders recorded in this study accounting for 13.4%. There were no statistically significant differences in occurrences of abnormalities at different age groups and areas of origin ($P>0.05$). However, there was significant difference ($p<0.05$) between pregnant and non-pregnant camels with higher occurrence in the latter groups. From

camels with endometritis 83% were positive for different types of bacterial isolates. *Escherichia coli* (38.7%) *Staphylococcus* spp (35.5%), *Streptococcus* spp (25%), *Enteriobacter aeroginosa* (16.1%), *Corynebacterium* spp (12.7%), *Proteous* spp (9.7%) and *Salmonella* spp (6.5%) were bacteria isolates identified in this study. It can be concluded that it is common to detect clinical pathologies in reproductive tracts of dromedary camels. Considering the importance of *Camelus dromedarius* among the pastoral society of the country, there should be collaborative studies pertinent to reproductive health to improve production and productivity of in this species.

Key Words: Bacteriology, dromedary camels, endomtritis, gross lesions, histopathology, reproductive tract

3.1. Introduction

Anatomical abnormalities and pathological processes of female reproductive tract have also been reported as the main causes of infertility (Deen, 2013). On top of these, infectious diseases of the reproductive system of camels may cause complications resulting in poor reproductive performance leading to infertility and consequent loss of productivity (Adel *et al.,* 2012). Thus, identification of reproductive tract diseases is important, especially when we deal with genetically superior animals (Ali *et al.,* 2010). Interest in the camel's reproductive processes only began when its economic benefits became apparent (Yagil, 2006).

The reproductive efficiency of camel under natural pastoral conditions is low, due to short breeding season, late age of puberty and long gestation period (13 months) (El-Hassanein, 2003; Skidmore, 2005). Other factors contributing to low fertility in camels include age at first calving, low libido of male thereby reducing breeding

opportunities and late postpartum oestrus (El-Hassanein, 2003; Al-Qarawi, 2005). For the diagnosis and management of reproductive performance and to circumvent the reproductive disorders, histopathological investigations and isolations of bacteria from uterus are critical (Mshelia *et al.,* 2012). Reproduction related problems are the main obstacle to production economics of camel and to genetic improvement, so its improvement is essential. There are several reports from other camel keeping countries, however, there has not been any research report on the pathological process at gross and histopathological levels and identify microbial causes of endometritis. Therefore, the intended objective of this part of the research is to investigate reproductive tract abnormalities of female dromedary camels and identify bacterial causes endometritis among dromedary camels slaughtered at Akaki slaughterhouse.

3.2. Materials and Methods

3.2.1. Study area and population

The study was conducted in Akaki municipal abattoir 20 KMs away from the center of Addis Ababa supplies which operates by slaughtering five to eight camels every day.

Female camels bought from Afar and Borana were the study animals. Afar camels from Afar National Regional State are smaller in size and extremely hardy and Borena camels from Oromia national regional state were of medium size and both served as pack, draught and dairy animals (Tezera and Kassa, 2012).

3.2.2. Study design

A cross sectional study design was conducted on 231 female camels examined for the pathological disorders and associated bacterial causes. Ages, physiological status and areas of origin were the variables of interest. Non probability sampling methods was used and all female camels slaughtered in the abattoir during every visit were examined for presence of any reproductive organ disorder.

3.2.2.1. Sample collection, transportation and processing

Everyday about 5-8 camels were slaughtered in the abattoir and 3-5 of them were female camels. Reproductive organs were removed from the animal and examined one by one for any gross pathological disease and disorders. Gross lesion examination was performed according to VMTH (2009) which included lesion distribution, contour, consistency, texture, shape, size and color.

Tissues with lesions were sampled for histopathology and bacteriological examination. The parts with lesions were cut to the size of 2-3 cm and put in the universal bottle containing 10% buffered formalin which stabilizes the tissues. The samples were transported to the National Animal Health Diagnostic and Investigation Centre (NAHDIC) Sebeta for processing. For bacteriological examination, the surface of the tissue with the lesion was decontaminated by moderate hot application using flame and then collected separately in sterile universal bottle, labeled and transported to NAHDIC in icebox following Quinn *et al.* (2004).

3.2.2.2. Histopathology procedures

Tissue processing was conducted following the procedure described by Talukder (2007) trimming, fixing in 10% buffered formalin, dehydrating in ascending grades of alcohol, clearing with xylene and impregnating with molten paraffin wax. Then, the tissues were sectioned at 5μm thickness, spread on warm water bath and were attached to albuminized glass slides. The slides were incubated at 60^0C to avoid paraffin wax followed by deparaffinization in three changes of xylene, rehydrating in descending grades of alcohol and staining with hematoxylin and eosin. Finally, the slides were examined under microscope using (10x, 20x, and 40x) magnification and photomicrographs were taken.

3.2.2.3. Bacteriological procedures

All bacteriological procedures were performed according to Quinn *et al.,* (2004). The sample tissue was minced with sterile scissors and forceps; inoculated to brain heart infusion broth and aerobically incubated at 37^0C for 24hrs. After 24hrs, a loop-full of bacterial growth were streaked on 7% sheep blood agar and incubated aerobically at 37^0C for another 24hrs. Then, the plates were checked for presence of growth and examined for colony morphology, size, shape, and presence or absence of haemolysis. The colony was sub-cultured on blood and MacConkey agars. For primary identification catalaset, oxidase, oxidation fermentation, motility tests, and gram stain was conducted. Selective and differential media such as mannitol salt agar, Edwards medium, eosine methylene blue (EMB), xylose-lysine-desoxycholate (XLD) and salmonella shigella agar were used for the suspected samples from the primary test results. After 24hrs of incubation characteristic growth on selective medium was registered.

For further biochemical tests, a colony was inoculated in to brain heart infusion. IMViC test including indole test, methyl red, Vagues-prousker (VP) test and citrate test were performed. Coagulase test, aesculin hydrolysis, urease test, lysine test, and sugar tests like glucose, sucrose, lactose, maltose, xylose, trehalose, raffinose and mannitol, were used as secondary biochemical tests.

3.2.3. Data analysis

Data were recorded, checked and coded on Microsoft Excel spreadsheet (Microsoft Corporation) and STATA version 11 was used for descriptive analysis, Chi-square test and binary logistic regression analyses at 95% confidence level. Chi square test was used to determine presence of dependency between different variables and infection and pathological abnormality of the reproductive organs.

3.3. Results

3.3.1. Reproductive organ abnormalities and lesions characterizations

Of the 231 female camels slaughtered in Akaki abattoir and examined for gross pathological disorders 46 (19.9%) have gross pathology in their reproductive organs (Table 6). Thirty (17.76%) of them were from Borana camels and 16 (25.8%) from Afar camels. The occurrence of reproductive disorders by origin, age and pregnancy status was evaluated and only pregnancy status has significant difference (Figure 15). Association of gross lesion types with age, origin and pregnancy also evaluated. A total of 129 camels were pregnant during the study period.

Table 6: Gross reproductive tract abnormalities with their respective numbers and percentages among dromedary camels slaughtered in Akaki abattoir, Ethiopia

No.	Types of lesion	No of camels with gross lesion	Percentage (%)
1	Follicular cysts	8	3.5
2	Ovarian hypoplasia	1	0.4
3	Ovarian tumour (hemangioma)	2	0.9
4	Para-ovarian cysts	2	0.9
5	Infundibular cysts	1	0.4
6	Endometrial problems	31	13.4
7	Uterine cysts	1	0.4
Total		**46**	**19.9**

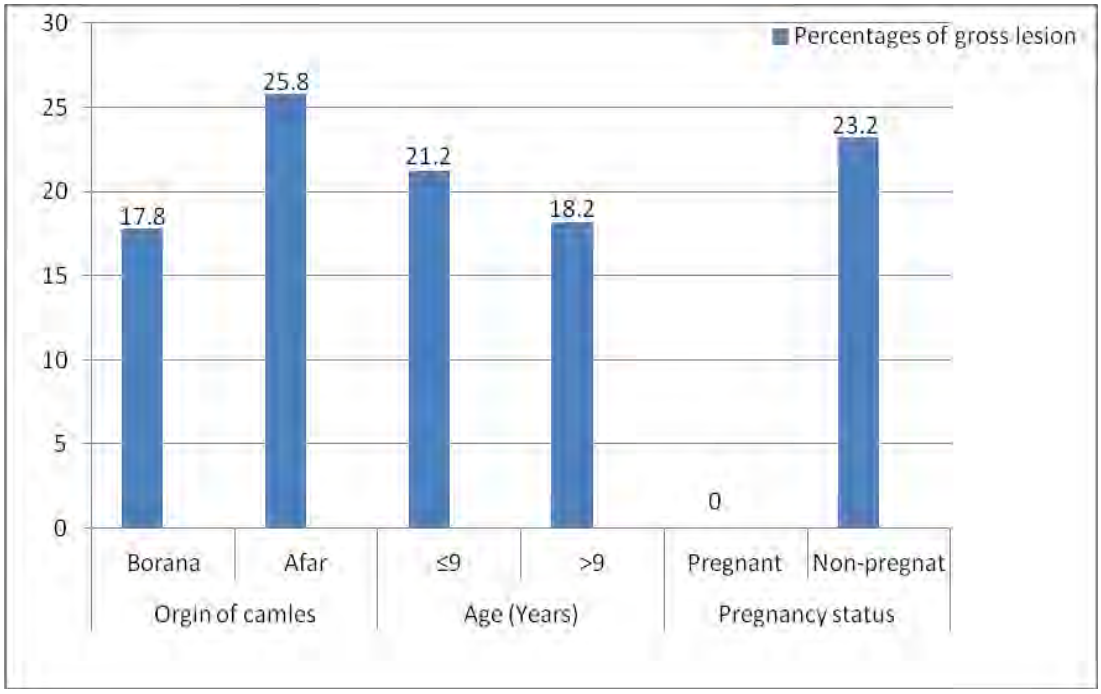


Figure 15: Distribution of reproductive tract disorder associated with origin, age and pregnancy status of dromedary camels slaughtered in Akaki abattoir, Ethiopia

Origin (X^2 =1.85, df=1, P=0.17); Age (X^2 =0.33, df=1, P=0.62); Pregnancy status (X^2 =9.57, df=1, P=0.002)

Follicular cyst was observed to be five times more in Afar camels than in Borana camels (OR=4.86, 95%CI, 1.12-20.96, P=0.034) and in pregnant animals no lesions were recorded. However, no significant difference was observed for other types of gross abnormalities (Table 7).

Table 7: Percentages and values of significance of gross reproductive tracts of *Camelus dromedarius* slaughtered at Akaki abattoir, Ethiopia

Factors considered with their respective X^2 and P-value, for each abnormality		Types of reproductive tract abnormalities in dromedary camels						
		Follicular cysts	Endometerial lesions	Uteri cysts	Infundibular cyst	Para ovarian cyst	Ovarian hypoplasia	Ovarian tumor
Origin								
	Afar	5 (8%)	8(12.9%)	1(1.6%)	0%	1(1.6%)	1(1.6%)	0%
	Borana	3 (1.7%)	23(13.6%)	0%	1(0.59%)	1(0.59%)	0%	2(1.18%)
	X^2	5.37	0.02	*	*	*	*	*
	P-value	**0.021**	0.89	2.7	1.0	0.47	0.27	1.0
Age (Years)								
	≤9	4 (3%)	20 (15.2%)	1(0.8%)	1(0.8%)	1(0.8%)	1(0.8%)	0%
	>9	4(3.4%)	11 (11.1)	0%	0%	1(1%)	0%	2(2%)
	X^2	*	0.79	*	*	*	*	*
	P-value	0.73	0.37	1.0	1.0	1.0	1.0	1.83
Pregnancy status								
	Pregnant	0%	0%	0%	0%	0%	0%	0%
	Non-pregnant	8(4%)	31(15.7%)	1(0.5%)	1(0.5%)	2(1%)	1(0.5%)	2(1%)
	X^2	1.38	5.97	*	*	*	*	*
	P-value	0.24	**0.015**	1.0	1.0	1.0	1.0	1.0

* = 2 and more than 2 cells have expected counts less than 5 and thus X^2 was not computed

3.3.2. Ovarian abnormalities of dromedary camels

3.3.2.1. Ovarian hypoplasia

Ovarian hypoplasia was diagnosed in one camel slaughtered in the abattoir during the study period. It was bilateral and the ovary was very small, oval in shape and measured 1.5 cm X 1.1 cm and 1.7 cm X 1.4 cm in length multiplied by breadth for

left and right ovary, respectively. Grossly the surface was almost smooth and the follicles were neither seen by naked eye nor palpated externally. Histopathologically the follicle were very reduced in number, not organized, blood vessels were congested in the center of the follicles and instead of forming follicles of different types, cells were simply aggregated in to focal area. Hyperplasia or excessive proliferation of stoma cells and fibroid connective tissue was also detected (Figure 16 A and B).

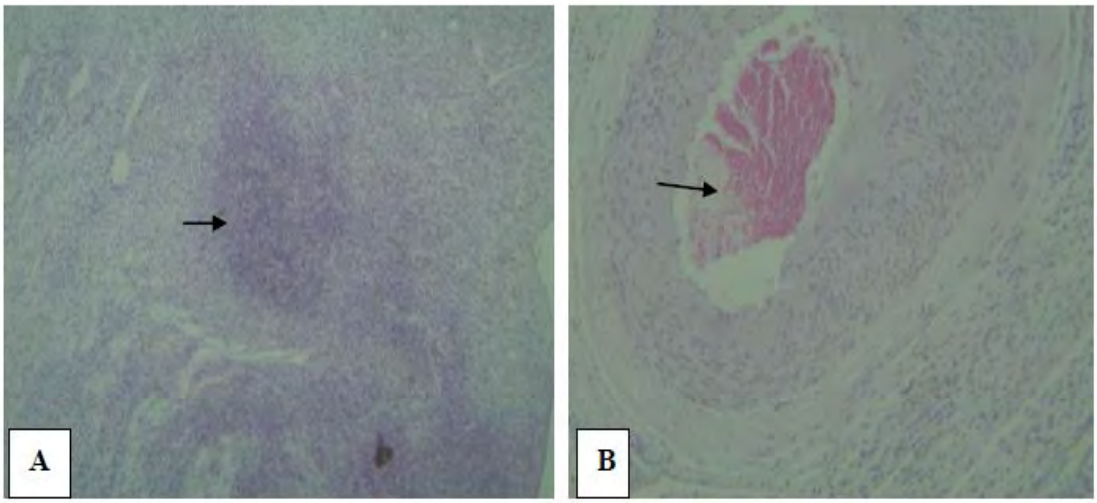


Figure 16: Hematoxyline-Eosine (H&E) stain of atrophied ovary. (A) hypoplastic ovary showing aggregation of cells (arrow) in one area without follicle formation, (B) hypoplastic ovary with congested blood vessel (arrow) seen in the center of less developed follicle (40x)

3.3.2.2. Follicular cysts in dromedary camels

Follicular cysts were detected in 3.5% of camels examined. These cysts contained clear fluid, enclosed in thin layer of connective tissue and granulosa cells. These cysts were externally pale and well vascularized. The sizes of these cysts ranged

between 23mm-36mm and all of them were on the left side (Figure 17A). Histologically cysts were characterized as fluid in the center, in which ovum was completely lost and only the fluid seen from the center. Thin layer of granulosa cells line the fluid; vascularized connective tissue layer bound the granulosa cells, theca interna and externally degenerating theca externa cover the entire cyst (Figure 17B).

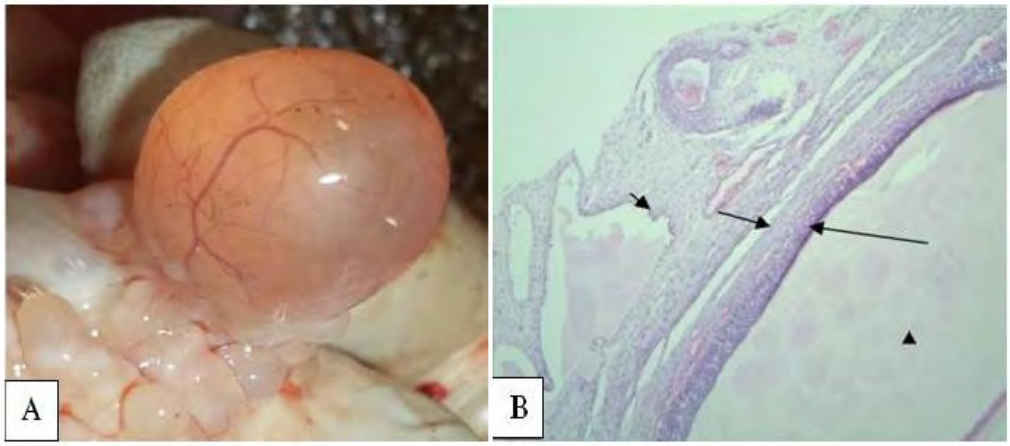


Figure 17: Follicular cyst of dromedary camels. (A) Gross, (B) histologic features (H&E stain) short arrow=theca externa & congested blood vessels, medium arrow=theca interna, long arrow= thin granulosa cells) and (arrow head = proteinecious fluid (40x)

3.3.2.3. Ovarian tumor and para-ovarian cyst

Two of the non pregnant camels slaughtered during the study period showed unilateral ovarian tumors (hemangioma) on the left ovaries. Grossly they were well demarcated, firm in consistency, 1.1 mm X 0.8 mm in length and width, almost circular in shape and dark-red in color. The outer surfaces of the neoplasms were smooth with prominent vessels and blood oozed from the cut surfaces (Figure 18A).

The remaining ovarian tissue was pale to gray and various numbers of corpus lutea and follicles were found in the ovaries.

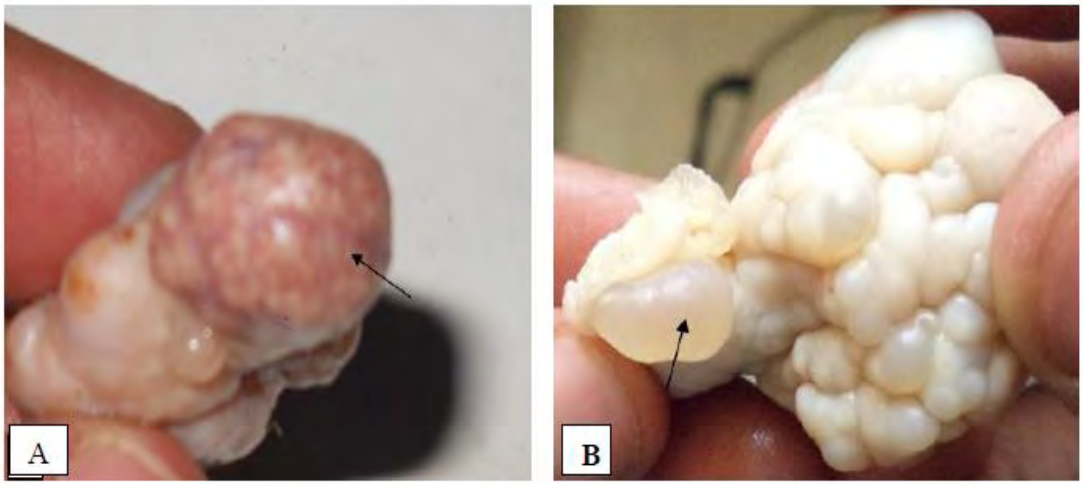


Figure 18: Macroscopic features of ovarian tumor (A) and para-ovarian cyst (B) in dromedary camels

Para-ovarian cysts are those cysts located near the ovary attached to the broad ligament and identified in two camels slaughtered. They were detected unilaterally only on the left sides of the ovary. In one case two cysts were appeared simultaneously on the ovary and in other case only one cyst was found on one side of the ovary (Figure 18B).

Histopathological examination verified the tumor as hemangioma. The neoplasms were composed of congested blood vessels. The vessels were elongated filled with blood and in some other vessels there were empty spaces or edematous fluid filled. The vascular spaces were separated by connective tissue stromal cells. There were also focal areas of cellular pigmentation and a few numbers of macrophages in the stromal cells (Figure 19A and B).

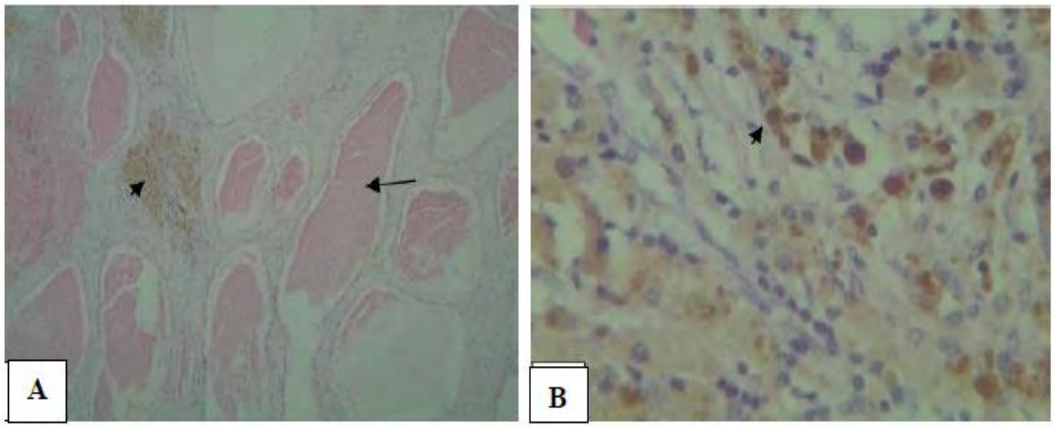


Figure 19: Histopathology of hemangioma in dromedary camels with H&E stain. Different pigmentation (long arrow) congested blood vessels (A) and (short arrow) stromal cells pigmentations (B) (40x)

3.3.3. Uterine abnormalities and lesion characterization

3.3.3.1. Uterine and infundibular cysts

Uterine serosal cyst was found in 1 (0.5%) of the examined uterus. This cyst was developed on the left side of the uterus, contains clear fluid and measured about 10mm in size (Figure 20A). Infundibular cyst was a clear serous fluid filled cyst which measured about 32mmx24mm of length and width. The wall of this cyst was externally thin, pale and clear-serous fluid was oozed during incision (Figure 20B).

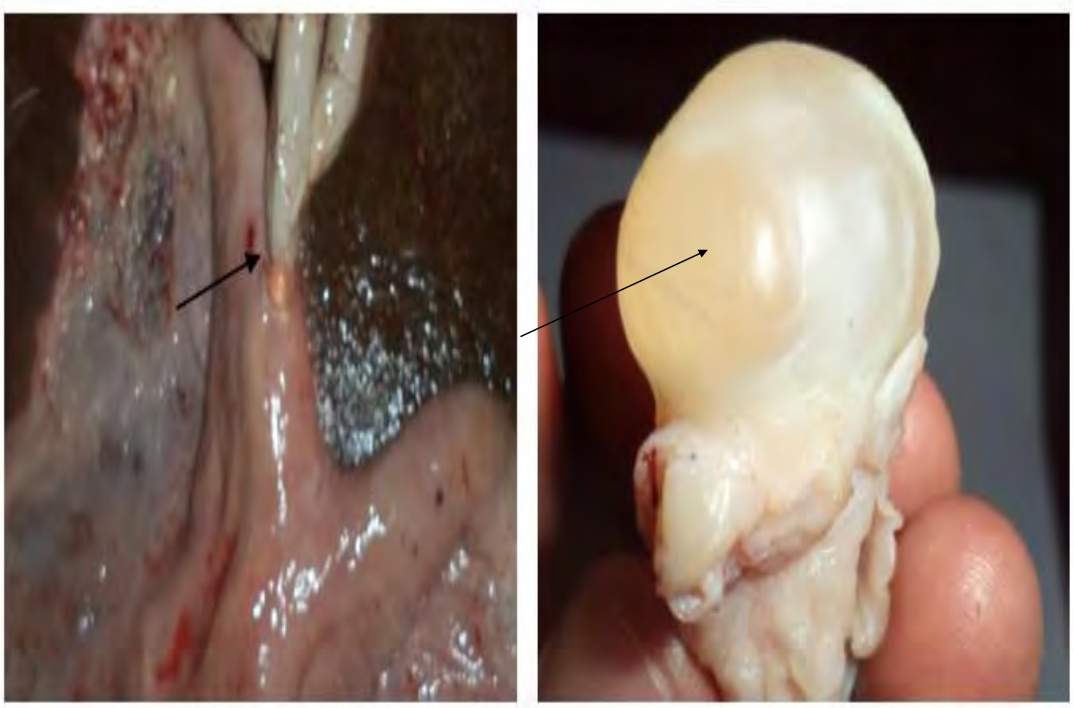


Figure 20: Macroscopic appearances of uterine serosal cyst (A) and infundibular cyst (B) in dromedary camels

3.3.3.2. Endometrial lesion

Uterine hemorrhages and endometrial congestions were found with high incidence rate than other pathological cases accounting for 13.4%. Of which 5.2% and 6% were acute and chronic endometritis respectively. Grossly these uteruses were congested in some parts of the endometrium, and in other cases the congestion and pin point hemorrhages were distributed over the entire parts of the endometrium, serosal parts of the uterus, including the body and horns of the uterus, even in some cases extended to cervix and vagina (Figure 21A).

In acute endometritis grossly congestion of the endometrium and enlarged (increased in width) uterus was observed. Histologically hemorrhage and infiltration of the polymorphonuclear cells (neutrophils) in endometrial layer of the uterus was detected (Figure 21B & C). In case of chronic endometritis microscopically the periglandular cuffing of lymphocytes, infiltration of macrophages, hyperplasia of endometrial glandular cells, suppuration with infiltration of neutrophiles and formation of edema in myometrium were detected (Figure 22A, B & C).

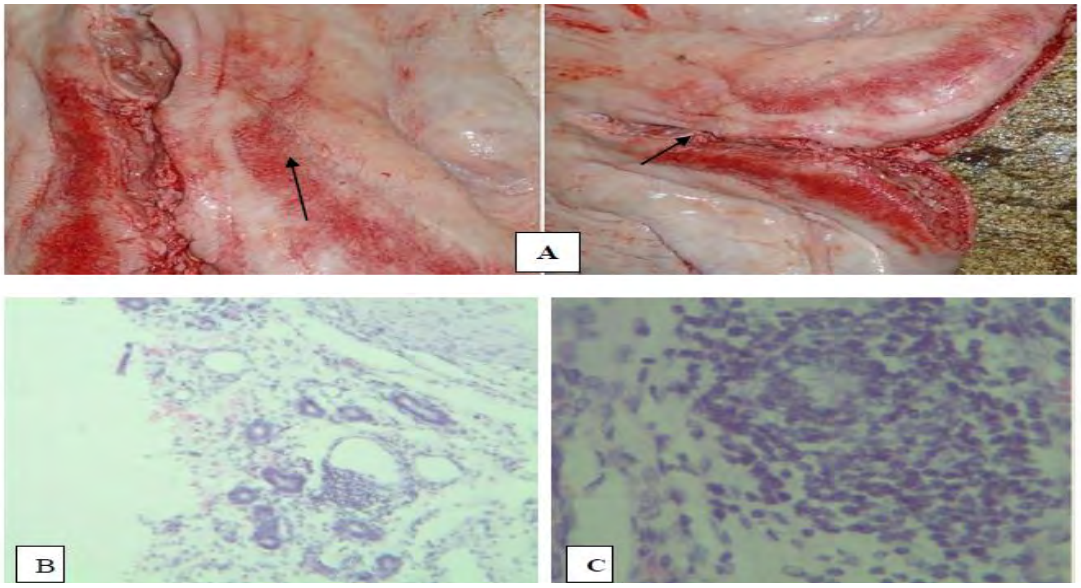


Figure 21: Gross pathology of endometrium with hemorrhage and congestion (A) Histologically, hemorrhage in endometrium (B) and periglandular cuffing of the lymphocytes in endometrium(C) (40x) in dromedary camels

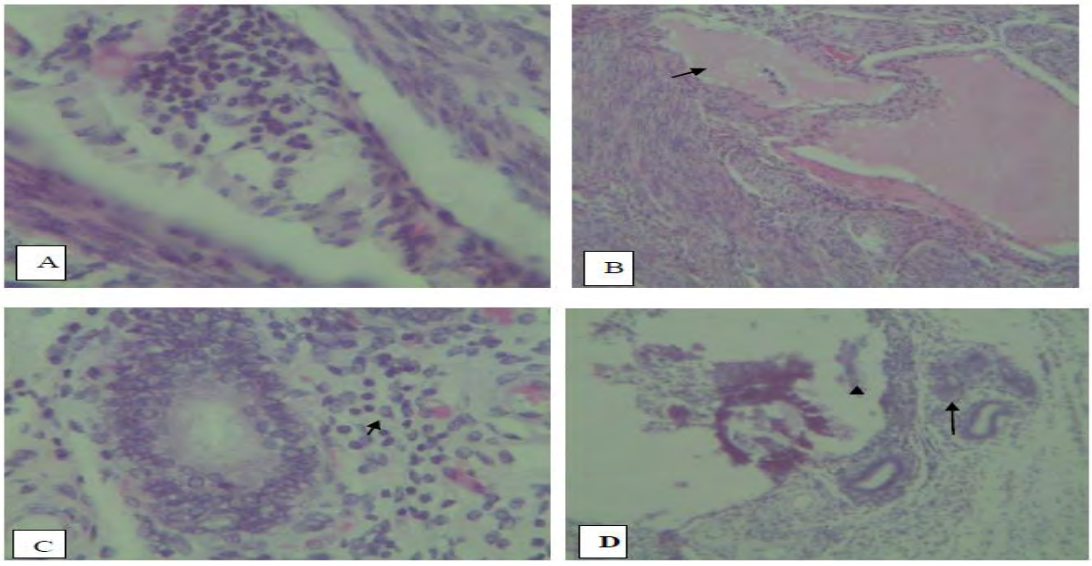


Figure 22: H&E stain showing neutrophile infilteration in myometrium (A), edema in myometrium (B), hyperplasia of glandular cells in which cells are enlarged and pale in color (dot arrow) and infiltration by plasma cell (long arrow) (C), Calcified substance with in the endometrium, and infiltration of the macrophages(D) (40x)

Endometrial calcification was found in one case. Grossly it was not easy to detect the uterine calcification case and only enlargement and tough consistency was detected. Histlogically calcification of the endometrium and infiltration of macrophages were clearly observed (Figure 22D).

3.3.4. Bacterial isolation

From 31 camels examined for endometritis, different types of bacteria were isolated in 26 (83%) camels and the main bacterial isolates were *Escherichia coli* and *staphylococcus* species (Table 8).

Table 8: Uterine bacterial isolates from *Camelus dromedarius* with endometritis

No.	Bacterial isolates	No of isolates	Percentages (%)
1	*Streptococcus* species	8	25.8
2	*Staphylococcus* species	11	35.5
3	*Salmonella* species	2	6.5
4	*Proteous vulgaris*	3	9.7
5	*Escherichia coli*	12	38.7
6	*Enterobacter aeruginosa*	5	16.1
7	*Corynebacterium* species	4	12.9

Except *Salmonella* species and *Proteous vulgaris* which were isolated only in acute endometritis, other bacteria were isolated from both acute and chronic endometritis.

Table 9: Association of bacterial detection with origin, age and pregnancy status of the slaughtered female dromedary camels at Akaki Abattoir

Risk factors	Categories	Number of examined camels	Number of positive camels	Percentage (%)	X^2 value	P-value
Origin	Borana	23	18	78.3		
	Afar	8	8	100	2.074	0.291
Age	≤ 9 years	21	19	90.5		
	> 9 years	10	7	70	2.1	0.296
Lesion Types	Acute endomeritis	12	12	100		
	Chronic endometritis	14	14	92.9	18.22	0.001
	Simple congestion	5	0	0		

There were no significant difference in bacterial isolates between age groups and origins of camels, but significant differences of bacterial isolation were seen among different types of lesions (Table 9).

3.4. Discussion

The results of this study demonstrated that reproductive organ diseases and disorders are important problems affecting the reproductive performance in female dromedary camels. The reproductive disorders were reported from different countries by (Mahmoud *et al*., 2011; Adel *et al*., 2012 and Mshelia *et al*., 2014) though no such

report from Ethiopia. Compared with previous reports the present study detects lower occurrences rates. Statistical significant difference in the occurrence of the reproductive organs disorder between the pregnant and non pregnant camels ($P < 0.05$) was observed since less likely to find abnormalities in pregnant animals. The number of pregnant animals slaughtered during the study period was a129.

Pathological lesion of endometrium was the common reproductive problem reported in previous studies. The present study also determined endometritis as a major reproductive organ abnormality followed by follicular cysts. However, the incidence of acute endometritis in the present study is lower than the reports in Saudi Arabia by Adel *et al*. (2012). The microscopic finding of the chronic endometrits was similar to the reports in Egypt by Shawky *et al*. (2004). A very large incidence of the endometritis (45.9%) was also reported in the Saudi Arabia by Ali *et al*. (2010). These variations might arise from differences in management system and veterinary services as well as nutrition and physiological differences of the camels at the different study countries. Over breeding, postpartum complications and unsanitary gynecological manipulation were the possible contributing factors to uterine infection in dromedary camels (Tibary *et al*., 2006) though it is not the case in Ethiopia as most of the lesions are in younger animals and the pastoralists don't allow their animals to over breed. Aggressive mating during the ''wrong'' phase of follicular developmental has been reported as a cause of severe uterine inflammation (Tibary *et al*., 2001; Vaughan and Tibary, 2006). Postpartum complications, unsanitary gynecological manipulations and errors include breeding with a young male, overuse of males, and lack of verification of intromission during copulation (Tibary and Anoussi, 2000; Ali *et al*., 2010) as causes of endometritis.

The total incidence of the ovarian disorders in this study was 4.8% including ovarian hypoplasia, ovarian hemangioma and ovarian follicular cyst lower than the finding by Adel *et al*. (2012) and by Shawky *et al*. (2004) with 17.14% and 40%

respectively. The differences could be attributed to nutritional, seasonal and genetics (Adel *et al*., 2012). Individual differences in identifying the pathologies might also be sources of differences among different reports.

The statistically significant difference in occurrences of follicular cysts between Borana and Afar areas might be due to variation in breeding practices of camels from the two areas as camels are induced ovulators. Adel *et al*. (2012) justified the probable variation in the occurrences of ovarian cysts is due to variation in the heat stress. This supports the report of the present study as the two areas are different in their temperature. Ovulation failure is caused by inadequate LH release in response to copulation (Skidmore and Adams, 2000; Kaufmann, 2005) and this could also be one of the factors for higher rate of incidences of follicular cysts among dromedary camels. The finding of ovarian follicular cysts of the present study was in agreement with the report in the eastern region of the Saudi Arabia by Adel *et al.* (2012) and slightly lower than in Kalyoubia, Egypt by Shawky *et al*. (2004) and higher than report from Iran by Nourani and Khodakaram-Tafti, (2004). The maximum size of follicular cyst reported by Adel *et al*. (2012) in Egypt is larger than reported in this study. The variation in the size of the follicular cyst could be due to age differences of the cysts.

The bilateral ovarian hypoplasia is comparable to report of Shawky *et al*. (2004) but lower than the reports Adel *et al.* (2012). The incidence of ovarian hemangioma in this study is in line with Shawky *et al*. (2004) and higher than Adel *et al*. (2012). The exploring of the causes of ovarian tumor needs further study. The Para-ovarian cyst report in the current study is lower than reports of Shawky *et al.* (2004) and Nourani *et al*. (2004) but higher than Adel *et al*. (2012). The size of the cysts presented in this report is lower than previous reports (Shawky *et al*., 2004). The infundibular cyst previously reported in Saudi Arabia by (Mahamoud *et al*., 2011) and (Adel *et al*., 2012) are higher than the current report. The size of the infundibular cysts reported

in this study is also lower than the size of the report from Saudi Arabia (Al-Eknah and Ali, 2001). In all of the reports, the cause of infundibular cysts is still not clear. The presence of cysts on the uterine serosa and broad ligament was in agreement with the finding of Skidmore and Adam (2000). Calcification in the endometrium is the first report in dromedary camels. Calcium salts, usually in the form of phosphates or carbonates, may be deposited (calcium buildup) in dead, dying, or normal tissue (Donnald and Zachery, 2002).

The 45 bacteria of 7 genera isolated from the endometritis in this study, is in line with the reports of Mshelia *et al*. (2014). The bacteria colonizing the reproductive tract of the female *Camelus dromedarius* have been incriminated as major causes of reproductive disorders in this species (Mshelia *et al*., 2014). These are regarded as the most important cause of infertility in domestic species. The reports of *Escherichia coli* from chronic endometritis and isolation of the *Proteous vulgaris* in acute endometritis in this study was similar with the report of Adel *et al*. (2012). Similarly the isolation of *Staphylococcus* species, *Corynebacterium* species and Salmonella *species* from endometritis are in line with the reports of Yagoub (2005) from Sudan.

Isolation of the *streptococcus* from endometritis is in line with reports of Abdel-Aziz and Nasser (2013) and Tibary and Anouassi 2000 also reported *Enterobacter aerogenosa* and *Salmonella* species. Evidence implicating bacterial infection as a cause of endometritis has been reported by Mshelia *et al*. (2014). The role of these bacteria as a cause of endometritis and infertility in dromedary camels needs further investigations.

CHAPTER FOUR

This chapter is based on the manuscript submitted for publication: Simenew K, Fekadu R., Tesfaye S. T., Tesfu K., Fufa D., Sentsui H. Serology of Viral Diseases causing Reproductive Failures among Camels (*Camelus dromedarius*) in Ethiopia *(Submitted to Journal of Veterinary Medical Sciences).*

4. SEROLOGY OF VIRAL DISEASES CAUSING REPRODUCTIVE FAILURES AMONG CAMELS (*CAMELUS DROMEDARIUS*), ETHIOPIA

ABSTRACT

Serological surveys were performed among camels in Ethiopia to examine antibodies to viruses which infect ruminants. The laboratory work was conducted in Japan at Nihon University. A total of 100 sera were collected from camels in various parts of Ethiopia in 2013. The present study showed several viruses that relate to the abortion of the ruminants were prevalent in Ethiopia. Bluetongue virus antibodies were especially detected at a high rate. Antibody to Akabane virus and Japanese encephalitis virus were also detected from about 40% of camels, though their antibody titers were rather low. The ratio of antibody positive camels to Ainovirus, Chuzanvirus and bovine viral diarrhea virus were less than 20%. Only one camel had antibody to bovine herpesvirus 1. As to the viruses which do not relate to abortion, bovine parainfluenzavirus type 3 infections were prevalent and antibody to parapoxvirus was also detected. The present study indicated the possibility that bluetongue virus could participate in the abortion of the camels in Ethiopia, though it

were not clear if the positive reactors were due to the special serotype of bluetongue virus infection or other serologically related viruses.

Key words: Abortion, blue tongue, dromedary camels, viral serology

4.1. Introduction

In Ethiopia they support more than 10 million pastoralists (Tezera *et al*., 2010; Bekele *et al*., 2002) and contribute 41% of the milk and 25.6% of the meat produced annually in the country. The camels occupy a pre-eminent position among the domesticated animals of hot, arid, and sandy regions. The camels have been bred owing to the extraordinary power to withstand thirst and hunger for long duration in the most inhospitable ecological conditions (Al-Dahash and Sassi, 2009). Despite the high population of camel and its extremely considerable importance in Ethiopia, it has not received adequate attention from research and development institutions as well as from policy makers. However, it is expected from the researchers and other respective bodies to act in a well organized manner to increase production and productivity of camels in the country as they have tremendous contribution in the daily livelihood of pastoralists and for the national economy at large. Reproduction related problems are the main obstacle to production economics of camel and to genetic improvement, so its improvement is essential. No previous studies were done in Ethiopia related to viral causes of reproductive failures. Therefore, this research is designed to study viral causes of reproductive failures in Ethiopian dromedaries using serology.

4.2. Materials and Methods

4.2.1. Study areas and study population

Selected districts of Afar and Somali regional states are the study areas. Study districts are selected on the bases of camel population, willingness of the camel owners to participate in the study and conveniences for transport as well as herd health report of abortion and infertility.

Camels in the selected study districts of the respective regions were the study animals. The main target to collect sera samples were animals with history of abortion and herds of reproductive infertility problems claimed by camel owners.

4.2.2. Sample collection, storage and processing

Blood samples were collected from 100 female camels using plane vacutainre tubes and waited for hours to separate sera. Serum was decanted and stored at -20^0C. The stored camel sera were transported to Japan, Nihon University, College of Bio-resource Sciences for laboratory analyses. The transportation to Nihon University follows the rules and regulations by OIE/FAO for biological sample transportation (Animal quarantine inspection number NFIB070602-011).

4.2.2.1. Viruses

The viruses used for the virus neutralization (VN) tests and Agar Gel Immunodifussion (AGID) test are shown in Table 10. Each virus for the VN test antigen was cultivated using the cells, and culture fluids were harvested when cytopathic effects (CPEs) appeared in more than 80% of cells. They were centrifuged at 3,000 rpm for 20 minutes and supernatants were separated in small vials and kept at -70^0C until use. Infectivity of the stock viruses was preliminarily detected by inoculating the stock virus after serial 10-fold dilutions into the cultivated cells in 96-well multi-plates (Sakamoto *et al*., 1994).

Table 10: Virus antigens used for the serological tests

Virus	Abbreviation	Strain	Serological test used
Bovine herpesvirus 1	BoHV-1	758	VN
Bovine herpesvirus 2	BoHV-2	Minnesota	VN
Bovine parainfluenzavirus 3	BPIV3	BN-1	VN
Japanese encephalitis virus	JEV	JaGar01	VN
Akabane virus	AKAV	OBE-1	VN
Chuzan virus	CHUV	C31	VN
Bovine viral diarrhea virus	BVDV	Nose	VN
Getahvirus	GETV	Sakai	VN
Ibaraki virus	IBAV	NO1	VN
Ainovirus	AINOV	95M46	VN
Bluetongue virus	BTV	Y-39	AGID
Bovine popular stomatitis virus	BPSV	Chiba	AGID
Bovine leukemia virus	BLV	FLK-BLV	AGID

4.2.2.2. Cells

Two cell lines and primary bovine fetal muscle cells were used for virus neutralization tests and virus cultivation. MDBK cells, originated from bovine kidney, were used for bovine herpes virus 1 (BoHV-1), bovine herpesvirus 2 (BoHV-2) and bovine popular stomatitis virus (BPSV). HmLu cells, originated from hamster lung, were used for Japanese encephalitis virus (JEV), Akabane virus (AKAV), Aino virus (AINOV), Chuzan virus (CHUV), Getah virus (GETV) and Ibaraki virus (IBAV). Primary fetal bovine muscle (FBM) cells were prepared by standard tissue culture methods and used within10 passages for Bovine viral diarrhea virus (BVDV). HmLu and MDBK cells were cultivated with Eagle's minimum

essential medium (MEM) supplemented with 5% fetal calf serum and 0.3% triptose phosphate broth (TPB). BFM cells were cultivated with Eagle's MEM supplemented with 10% fetal calf serum. For the preparation of bovine leukemia virus (BLV) antigen for immmunodiffusion testing, fetal lamb kidney cells persistently infected with BLV (FLK-BLV) were cultivated in medium consisting of 5% FCS and 95% Eagle's MEM and the culture fluid was used (Kuroda *et al*., 1999). All culture media were supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin.

4.2.2.3. Agar gel immunodiffusion test

The culture fluid of FLK-BLV and HmLu cells infected with Ibaraki virus were concentrated using ammonium sulfate, transferred in a dialysis tube and dialyzed, and further concentrated in polyethylene glycol. The concentrated virus fluid was treated with triton X-100 (final concentration was 1.0%) and used as an antigens for each virus antibodies. The procedures are described in OIE (2004 and 2010). MDBK cells infected with parapoxvirus were detached from the culture bottle using a rubber policeman, collected in a centrifugation bottle and washed 3 times in PBS by low speed centrifugation. The sediment cell pellets were suspended in a small volume of PBS, treated with triton X-100 (final concentration was 1.0%) and used for antigens. AGID tests were also performed according to the OIE manual. The gel consisted of 0.8% noble agar, 8.5% NaCI and 10mM phosphate buffer at pH 7.4. The wells were 5mm in diameter, and six circumferential wells were placed at a distance of 3 mm from the central well. The central well was filled with the antigen and two opposite exterior wells were filled with positive control serum. Serum samples were placed in the remaining four wells. The gel diffusion plate was allowed to stand room temperature for 48 hour and precipitation lines were observed. The virus types with their respective abbreviations are presented in table 10.

4.2.2.4. Virus neutralization test

The sera were titrated in two duplicate wells across a 96-well microplate from an initial 1/4 dilution. Then 0.05 ml samples of serial 2-fold serum dilutions were mixed with an equal volume of virus having 200 TCID50/0.1ml in 96-well microplates and incubated at 37^0C for 60 minutes. Cell suspensions were added to each well and incubated at 37^0C until CPE appeared in virus control cells. The VN antibody titer was expressed as the reciprocal of the serum dilution that inhibited CPE. Samples that were positive for antibodies at a dilution of 1:8 or greater were considered as seropositive (Finney, 1964; OIE, 2010).

4.3. Results

At first, about 100 samples with relatively large volume were selected from all samples and subjected to the serological survey. The results of the serological tests to each virus are presented in table 11.

Antibodies to BoHV-1, BoHV-2 and BLV were almost negative among the camel. Since BHV-1 infects various animals including horses experimentally, the camel would be infected with this virus. However, since the cattle might not be distributed in the desert zone and kept together, the camel would have low opportunity to contact with the cattle. This could be the reason why only one antibody-positive camel was detected.

Since bovine mammilitis virus and pseudolumpy skin virus are classified in BoHV-2, we thought they might be cross react each other. However, positive reactor to BoHV-2 Minnesota strain was not found. Since there is enough information about the serological relation between these two viruses, present results could not provide the conclusion that the camel is not infected with pseudolumpy skin disease virus.

Furthermore, since it can be transferred by blood-feeding insects mechanically, infection from cattle to camel is not possible in the desert zone where cattle was not near and blood on hypodermic needle of insect dries soon.

Table 11: Distribution of antibody-positive animals

Virus (Abbreviation)	Antibody titers						Total of positive serum (%)[1)]
	< 4	4	8	16	32	>32	
BoHV-1	99	0	1	0	0	0	1.0
BoHV-2	100	0	0	0	0	0	0.0
BPIV3	36	13	10	13	8	20	51.0
JEV	33	26	21	11	2	4	38.4
AKAV	37	17	20	15	5	6	46.0
CHUV	47	28	11	6	0	0	18.5
BVDV	72	0	2	4	3	5	14.7
GETV	75	7	4	2	0	4	10.9
IBAV	11	29	14	9	8	24	57.9
AINOV	61	18	5	2	3	5	20.0
BTV	8 [2)]			92 [3)]			92.0
BPSV	78			22			22.0
BLV	120			0			0.0

[1)] Antibody titers equal to or more than 8 were regarded as positive.

[2)] Antibody negative by AGID test.

[3)] Antibody positive by AGID test.

The rate of antibody-positive camel to BPIV3 was high, and those to BVDV and BPSV were moderately high. Because BPIV-3 can grow in many kinds of cells originated from various animals in vitro, the host range of this virus would be rather wide. There is also a possibility that PIV-3 originated from the camel may exist and cross react serologically with BPIV-3. BVDV cross react with hog cholera virus in pig and border disease virus in sheep in neutralization tests. Therefore, it is not

clarified that the antibody positive animals detected in this study are due to BVDV. A pestivirus which originated from the camel as a natural host may exist. Four viruses are classified in genus parapoxvirus and they have been isolated from various ruminants including the camel in Syria, and cross-react each other in immunodiffusion test. Therefore, it is quite likely that Ethiopian camel would be infected with the virus. The reason why an antibody-positive rate was low would be the sensitivity of the immunodiffusion test.

4.4. Discussion

The present study indicated that camels have antibodies to many arthropod-borne viruses that are prevalent among ruminant in Japan (Izumida *et al*., 1990). Therefore, it is quite likely that a natural transmission cycle between the camels and arthropods are formed in Ethiopia. Among the epidemics associated with the abortion among the ruminant and arthropods, AKAV, AINOV, CHUV, IBAV and BTV are important in Japan (Al-Dahash and Sassi, 2009). Domestic animals in Ethiopia are suffered by various kinds of arthropods such as ticks, mosquitoes, midges, lice and so on, in comparison with those in Japan. Therefore, an unknown infection cycle may be formed between camels and arthropods. It is thus necessary to consider the participation of arthropod-borne virus infections among camels for the control of their abortions.

The camel had antibodies to AKAV and BTV to a high rate among these viruses. AKAV infection in adult animals is subclinical but may lead to fetal losses, abortions, stillbirths and the appearance of weak dumb calves with skeletal defects of the limbs and vertebral column. The abnormal parturitions by AHAV infection in cattle are characterized by congenital arthrogryposis and hydranencephaly (Mobini *et al*., 2002; Kono *et al*., 2008). The cause of the reproductive difficulty among the

camel in Ethiopia would be elucidated by histopathological inspections on the fetus with abortions, stillbirths and abnormal parturition. However, antibody titers to AKAV among the camel were not high compare to the cattle which caused abortion or abnormal parturitions in Japan. Therefore, the camel might be infected with other virus which cross-react with AKAV in Ethiopia.

The present results indicated that BTV infection was prevalent among camel in Ethiopia though it is not clear if the positive reactors would due to the infection of BTV or other serologically related viruses including IBAV. Clinical signs of bluetongue in domestic and wild ruminants range from subclinical in the vast majority of cases to an acute febrile response characterized by inflammation and congestion, leading to edema of the face, eyelids and ears, and haemorrhages and ulceration of the mucous membranes (Mobini *et al*., 2002; Kelling, 2007). Many serotype of BTV have been reported all over the world and the pathogenicity are greatly variable according to the serotype. It is reported some BTV strain cause abortion and an abnormal parturitions. Therefore, there may be BTV strain which has high pathogenicity on the camel and BTV would participate to the reproductive difficulty among the camels in Ethiopia.

The antibody to GEV was detected, though the rate of positive camel was rather low. The breeding of pigs, a carrier of GEV, is not common in Ethiopia, though wild boars live there. Therefore, the conditions for an epidemic of GEV are insufficient. On the other hand, the antibody to JEV was also detected more high rate, but the seropositive camel did not have high antibody titers to JEV. West Nile virus (WNV), which cross-reacts with JEV, is present in Africa and the reservoir is birds (Hanna *et al*., 1999; Erlanger *et al*., 2009). It needs to be clarified in the future whether the JEV antibody detected in camels in this study is due to JEV infection or to other viruses such as WNV having a common antigen to JEV.

The present study provides information on the current prevalence of the representative viral diseases in Ethiopia among camels with history of abortion To recognize the ecology of these viruses and control camel diseases in Ethiopia, further large scale epidemiological investigations is required and viral genome identification should proceed.

CHAPTER FIVE

This last section is adapted from the manuscript submitted for publication: Simenew K., Fekadu R., Tesfaye S. T., Tesfu K., Juri V., David O.O., Stelletta C. Testicular cytological profiles of apparently healthy dromedary camel (*Camelus dromedarius*) bulls *(Submitted to Journal of Animal Reproduction Science).*

5. TESTICULAR CYTOLOGICAL PROFILES OF APPARENTLY HEALTHY DROMEDARY CAMEL (*CAMELUS DROMEDARIUS*) BULLS

ABSTRACT

The application of testicular fine needle aspiration (TFNA) in human and other animal species has been proven for the evaluation of cytological profiles and for fertility test. However, in dromedary camels the use of TFNA is not yet reported. Early diagnosis and determination of fertility status of dromedary camel bull is the base for ultimate reproductive performances. This study evaluated testicular cytological profiles of apparently healthy dromedary bulls, and compared cytological proportions between TFNA and imprint smears in different seasons. Twenty six (18 non-rutting and 8 rutting seasons) dromedary bulls 6-12 years old slaughtered at Akaki, Addis Ababa abattoir were studied. Pairs of testes from slaughtered bulls were collected and TFNA and impression smears were obtained. A 21 gauge needle attached to 20 ml syringe was used to collect TFNA samples and 5 aspiration smears were prepared. A total of 312 slides (260 TFNA and 52 imprints) were examined. Each imprint smear slides had 3 imprints, thus a total of 416 for both seasons. Smears were stained using modified May-Grunwald Giemsa (mMGG) technique and

light microscope used to assess cellularity, morphology and quantification of the testicular cytology. Sertoli cells and spermatogenic cells including primary and secondary spermatocytes, spermatogonia, early and late spermatids and matured spermatozoa were identified and counted. Leydig cells were also observed in few slides. The different cytological indexes considered were spermatic index (SI), Sertoli cell index (SEI) and the relationship between SI and SEI indexes (SSEI) used to explore the ratio between mature spermatozoa and nursing cells, representing the ability of the testes to produce sperm cells. There were significant differences ($p<0.5$) between the rutting and non-rutting seasons among the spermatogenic and Sertoli cells except primary spermatocytes, early spermatids and SSEI. There was no difference ($p>0.05$) between TFNA and imprint smears of the testicular cells except for Sertoli cell count and its index (SEI). Filarial worm larvae were demonstrated in the TFNA smears of 4 animals (1 from breeding season and 3 from non breeding season). The current findings suggest the use of TFNA in dromedary camel bull breeding soundness test and diagnosis of infections in dromedary camels. Further study to standardize the use of this technique at different species might help a lot in the reproductive performance evaluation.

Key words: Dromedary bulls imprint smears, rutting season, testicular fine needle aspirations

5.1. Introduction

The pastoralist themselves select their breeding bull using physical appearances and progeny history. Infertility problems among dromedary camels (Skidmore, 2005; Tibary *et al.,* 2006) have been reported to be high, and there have never been any proper diagnostic and intervention methods devised against such problems so far among the pastoral dromedary camels. Devising proper clinical examinations and

testing for breeding soundness in dromedary camel bulls are of paramount importance.

Assessment of spermatogenesis is an important component in the evaluation of male fertility in human and animals. Testicular fine needle aspiration combined with the introduction of Intra Cytoplasm sperm Injection (ICSI) has revolutionized the management of male infertility in recent years (Papadimas *et al*., 2004; Mikos *et al*., 2007). Infertile men with severe spermatogenesis disorders can give birth to their own children, whereas only a few years ago the same group of men had only to choose between sperm donation and adoption (Mikos *et al*., 2007). Testicular fine needle aspiration cytology has been implemented for human and adopted to veterinary medicine in very recent days (Stelletta *et al.*, 2011; Pintus, 2012, Vencato *et al*., 2014).

Although the main techniques for obtaining testicular parenchyma *in vivo* are open biopsy and puncture biopsy, these techniques are invasive and provoke severe and sometimes irreversible damage to reproductive efficiency of the males (Mahajan *et al*., 1999; Meng *et al*., 2001). Because of its minimal invasiveness, TFNA could be used repeatedly *in vivo* on the same individual without affecting reproductive activity (Leme and Papa, 2000; Westlander *et al*., 2001; Gouletsou *et al.*, 2010).

Application of TFNA in animals is so far very limited as compared to its age of inventions and the significant contribution of the technique. Vencato *et al*. (2014) strongly recommended the use of trans-scrotal ultrasonography and testicular fine-needle aspiration cytology for effective breeding soundness test in rams and this may be applicable in other animals and human too. Above all, the use of TFNA in male dromedary camels has never been reported and standardization of this technique for sure will have significant impact on assessment of dromedary bulls for breeding

soundness. Early diagnosis and determination of fertility status of the bull in dromedary camel is the base for the ultimate herd performances. It makes this technique very important over other species as one dromedary bull may mate more than 50 she-camels. Therefore, this research is intended to evaluate the effectiveness of testicular fine needle aspiration as clinical diagnostic technique for bull fertility.

5.2. Materials and Methods

5.2.1. Testicular cytological sample collection

Both TFNA and imprint smears were prepared from apparently healthy dromedary camel bulls slaughtered at Akaki Addis Ababa municipality abattoir during the months of June-September 2013 (out of breeding season), and the other groups during January and February 2013 (breeding season). Although the age of the bulls slaughtered ranged from 2-18, for the purpose of this typical testicular cytological study bulls of 6-12 years old were sampled. Younger ages and older ages of camels were intentionally excluded from the study due to their smaller and non-functional testes. A total of 26 bulls (18 from the non-breeding and 8 from the breeding seasons) were sampled and examined for testicular cytological profiles. The bulls were identified using numbers given by the butchers, and their ages were determined by dentition according to Dioli (2013). Both testes from individual slaughtered bull were carefully taken and TFNA and impression smears were made.

5.2.1.1. Testicular fine needle aspirations (TFNA)

The testicles to be aspirated were immobilized by handling as firmly as possible. A 21 gauge needle attached to a 20 ml syringe was used. The needle was introduced into the center of the testicle and strong negative pressure was applied on the syringe. Because cell population retrieved from testis is highly dependent upon the site aspirated, a representative sample was obtained by redirecting and moving the needle to several areas in the middle of the testis while maintaining the negative pressure. After the needle was observed to contain sufficient tissue for smears it was withdrawn and the negative pressure was relieved before the needle was finally out. The contents of the needle were expressed onto a slide and a smear prepared by placing another slide over the sample to spread the sample. The top slide was rotated about 45 degrees and lifted directly upward, producing a spread preparation with subtle ridges and valley of cells. Five slide smears were prepared from each testis.

5.2.1.2. Impressions smear

After aspiration, the testes were cut cross-sectional in to two equal halves and imprints were made in one slide at 3 places by touching the slide gently to the testis, followed by quickly air-drying.

5.2.2. Cytological staining procedures

The staining of the TFNA and imprint smear slides was conducted following Foresta (1993) as referred by Vencato *et al*. (2014). Briefly, the slides were stained with Modified May-Grunwald Giemsa (MGG) for 4 minutes and then rinsed with 1-2

drops of distilled water for 2 minutes; then the slides were stained with Diluted Geimsa (1:20) for 20 minutes and rinsed with distilled water. The slides were dried and observed under microscope for cellularity and then identification of testicular cells.

5.2.3. Microscopy and interpretation of testicular cytological preparations

Once the smears have been prepared, stained and dried, they were scanned at low magnification **(**10X objectives**)** to determine cellularity for evaluation. When the cellularity was recognized, magnification was increased to the 20X and 40X objectives. Then, cell morphologies were evaluated in detail with the 100X **(**oil-immersion**)** objective.

Quantitative and qualitative analyses were made on each smear. Nuclear and cytoplasmatic characteristics, staining characteristics, morphology and size were considered in order to identify the testicular cell populations. Differential counting of at least 200 testicular cells was undertaken on each smear to calculate the relative percentage of each cell population and the percentage of spermatozoa with respect to the total number of germ cells (spermatic index, SI). The ratio between the numbers of Sertoli cells per 100 spermatogenic cells was used to determine the Sertoli cell index (SEI) and the ratio of SI and SEI indexes (SSEI) was used to explore mature spermatozoa and nursing cells, representing the ability of the testes to produce sperm cells (Griswold, 1995; Romagnoli *et al*., 2009; Johnson *et al*., 2008). The typical features of each testicular cell types are presented in Table 12 and Figure 23 of the result section.

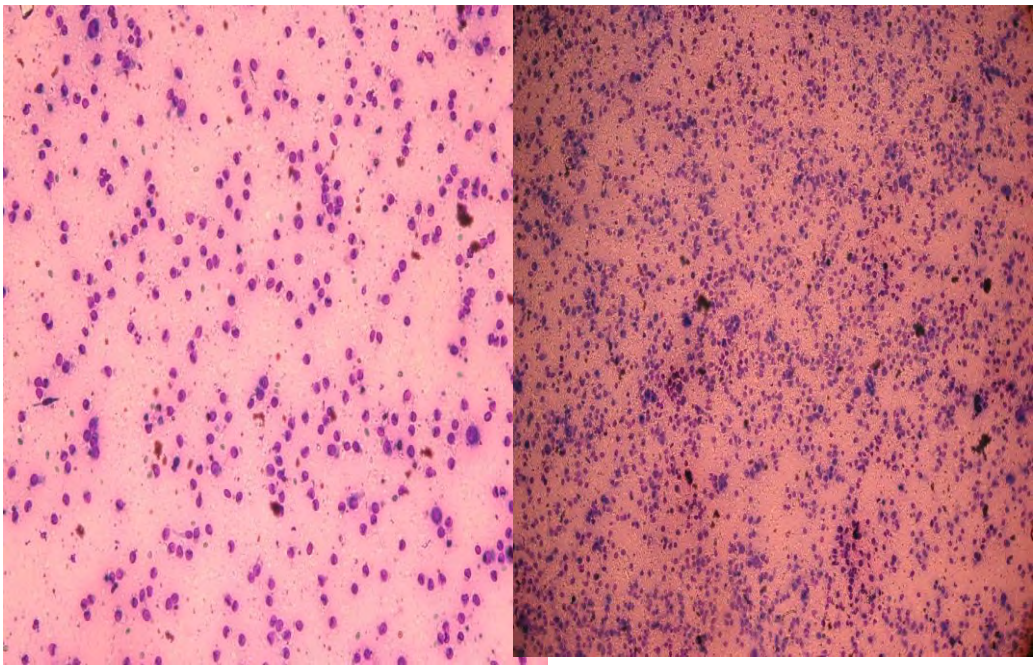
5.2.4. Statistical analyses

Statistical analysis was performed using statistical software SPSS version 20 (SPSS Inc, Chicago, IL, USA). One way ANOVA was carried out to analyze variations among cellular types of the two smears (TFNA and imprint), seasons (breeding and non-breeding) and sides of testes (right and left). Differences were considered significant at $p< 0. 05$.

5.3. Results

5.3.1. Morphometric characterization of testicular cells in dromedary camels

A total of 312 slides (260 TFNA and 52 imprints) were examined. However, on each imprint smear slides there are 3 imprints and makes the total smears 416 for both seasons. The testicular cells were identified and characterized accordingly as presented in tables 12.



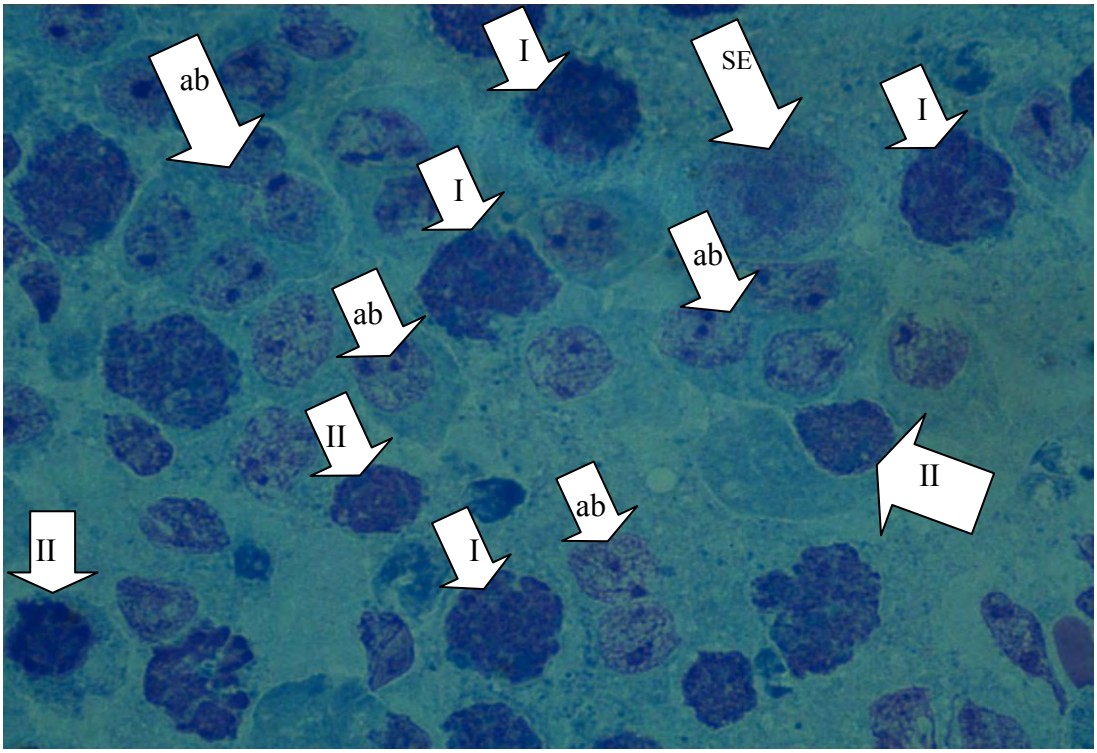
A B

Figure 23: Cellularity features of testicular fine needle aspiration (A) and imprint (B) smears after modified May-Grunwald Giemsa staining and examined under 10X magnification

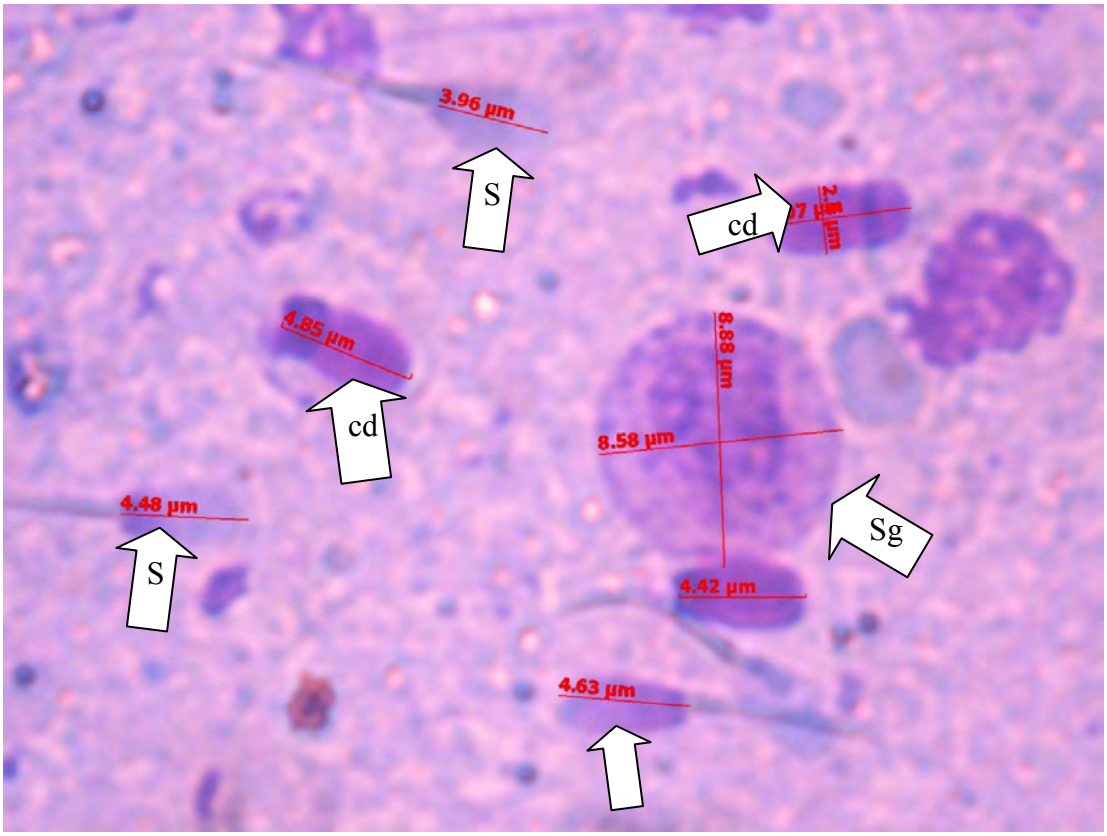
Leydig cells were the largest of all cells identified in this study followed by spermatoginia and primary spermatocytes. In addition to cell size, testicular cells were also characterize on account of their morphology, staining characteristics of their chromatin and cytoplasm, position of nuclei and cytoplamic content as shown in figure 24A-C.

Table 12: Performance characteristics of TFNA and imprint smears of dromedary camel bulls

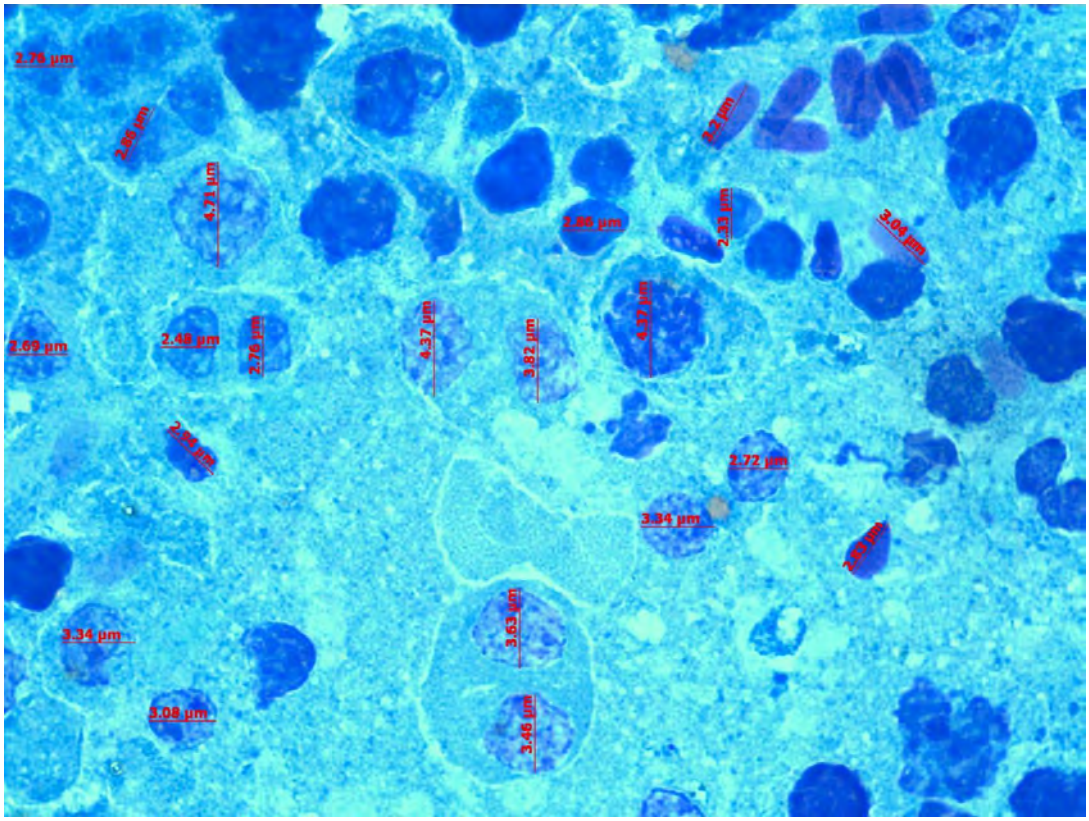
Ref. numbers	Types of testicular cells	Characteristics of cells
1	Leydig cells	They are the largest of all cells identified in the smears. They are large rounded cells contain central nucleus and the nucleus contains course chromatin
2	Sertoli cells (SC)	They are large; nucleus appears round or oval with finely granulated chromatin. Mostly spermatozoa are associated themselves with them. They are one of the easily identified testicular cells in the smears.
3	Spermatogonia (Sg)	Usually appear as naked nuclei with fine and homogeneous chromatin. Their nuclei are bigger than the nucleus of sertoli cell. The cytoplasm is scarce. The color ranges from pale to dark.
4	Primary spermatocytes (I)	They are round or oval in shape and large in size. The chromatin is clumped with coarse chromatin threads. The cytoplasm is very scanty.
5	Secondary spermatocytes (II)	Cellular diameter is almost half the sizes of those of primary spermatocytes. The chromatin structure is finely granular and these calls are rare to identify due to their short lifespan.
6	Early spermatids (ab)	Round shaped with smaller nucleus than secondary spermatocytes. The chromatins differ from uniformly fine granular to course granules like primary spermatocytes.
7	Late spermatids (cd)	There were both round and elongated nuclei late spermatids in these species. Elongated spermatids are characterized by more elongated and darker nuclei. Multinucleated forms are also common. The round forms are smaller in size than ab cells.
8	Spermatozoa (S)	They are easily distinguished on the basis of the oval-shaped head and the presence of the tail. It is not difficult to differentiate them from the darker nuclei elongated late spermatids.


ab
I
SE
I
I
ab
ab
II
II
ab
I
II

A


3.96 µm
S
cd
4.85 µm
cd
8.88 µm
8.58 µm
4.48 µm
S
Sg
4.42 µm
4.63 µm

B



C

Figure 24: Morphometric pictorial demonstration of Sertoli and spermatogenic cells in impressed slides (A) and in smears of TFNAC (B and C); SE-Sertoli cells, Sg-spermatogonium, I-primary spermatocyte, II-secondary spermatocyte, ab-early spermatid, cd-late spermatid, s-spermatozoa, MGG stain with 100X magnification

Table 13: Mean ± S.E.M., minimum and maximum testicular cells sizes of TFNA and imprint smears of apparently healthy dromedary camels

Testicular cell types	Mean ± S.E.M. (µm)	Minimum-Maximum (µm)
SE length	6.36±0.24	5.03-9.16
Sg length	7.90±0.46	6.24-8.88
I chromatin length	6.19±0.23	4.33-7.91
I cytoplasm	9.23±0.00	9.23
II length	4.35±0.86	3.94-4.76
ab length	3.74±0.09	3.08-4.74
cd length	2.80±0.10	1.80-3.59
S Head length	3.50±0.10	2.33-4.26
Lg cells length	12.67±0.00	12.67

SE=Sertoli, Sg= spermatogonium, I= primary spermatocyte, II=secondary spermatocyte, ab=early spermatid, cd=late spermatid, S=spermatozoa, Lg=Leydig cell

It is not easy to find Leydig cells or misidentified. However, in this study during the breeding season testicular cytology study it was possible for the researchers to come across with very few Leydig cells in dromedary camels as indicated in tables 12 and 13 as well as in figure 25.

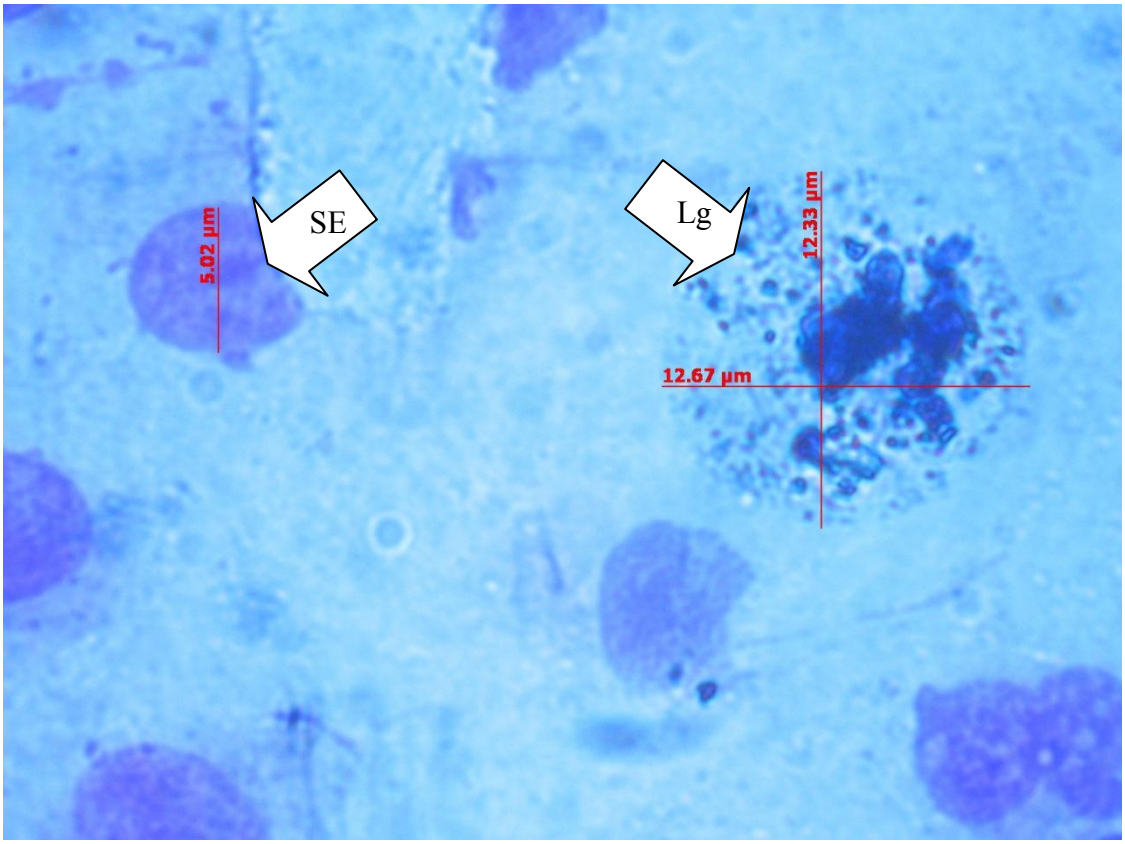


Figure 25: Leydig cell (Lg) in dromedary camel testis observed during the rutting season

5.3.2. Quantitative testicular cytology in dromedary camels

The relative percentages of testicular cytology profiles are presented for the non-breeding seasons (Figure 26 and Table 14) and breeding seasons (Figure 27 and Table 15).

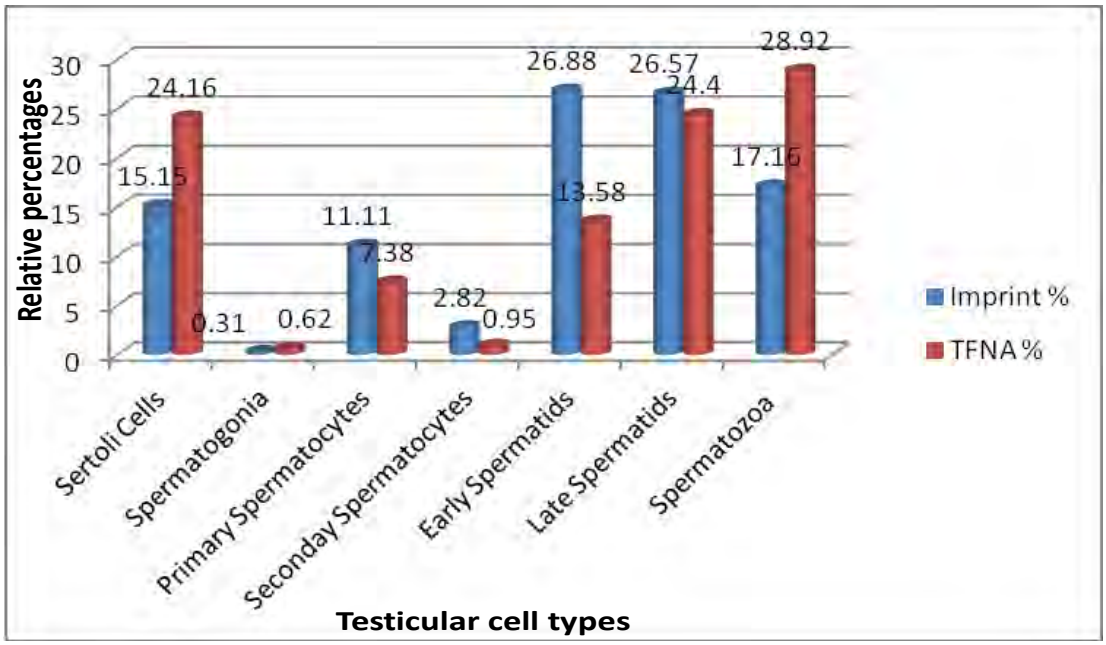


Figure 26: Relative percentage distributions of testicular Sertoli and spermatogenic cells in dromedary camel bulls during non-breeding seasons of imprint (blue) and TFNA (red) smears

During the non-breeding season of dromedary camels the abundance of testicular cells indicated spermatozoa, early spermatids, late spermatids, Sertoli cells, primary spermatocytes, secondary spermatocytes and spermatogonia in the order of decreasing proportion (Figure 26).

Table 14: Testicular cell types and indexes of TFNA and imprint smears (mean ± S.E.M.) of apparently healthy dromedary camel bulls (N=18) during non-breeding season

Testicular cell types and indexes	TFNA Smear		Imprint Smear	
	Right Testis	**Left Testis**	**Right Testis**	**Left Testis**
SE	62.02±3.89	46.48±2.76	39.56±5.03	28.41±3.5
Sg	[a]1.82±0.19	[a]2.09±0.19	[a]2.6±0.68	[a]1.5±0.5
I	[a]15.86±0.91	[a]17.06±1.08	29.00±2.75	37.24±3.00
II	[a]3.62±0.58	[a]3.89±0.61	7.38±1.02	7.88±1.11
ab	[a]29.63±1.7	[a]31.42±2.26	[b]66.06±5.49	[b]70.18±7.55
cd	[a]55.1±2.42	[a]54.63±1.89	[a]65.29±4.91	[a]54.94±4.2
S	[a]60.97±4.05	72.25±3.61	[a]37.74±5.56	46.65±6.13
SI	[a]35.71±1.8	38.21±1.96	25.47±6.37	[a]21.31±2.48
SEI	43.08±3.49	28.5±2.17	19.68±2.31	14.17±2.18
SSEI	[a]195±32.61	[a]212.44±21.84	[a]117.83±33.08	[a]225.22±49.34

SE=Sertoli, Sg= spermatogonium, I= primary spermatocyte, II=secondary spermatocyte, ab=early spermtid, cd=late spermatid, S=spermatozoa, SI= spermatic index, SEI=Sertoli cell index, SSEI=spermatic to Sertoli cell indexes; identical letters of same column indicate no significant differences ($p>0.05$),

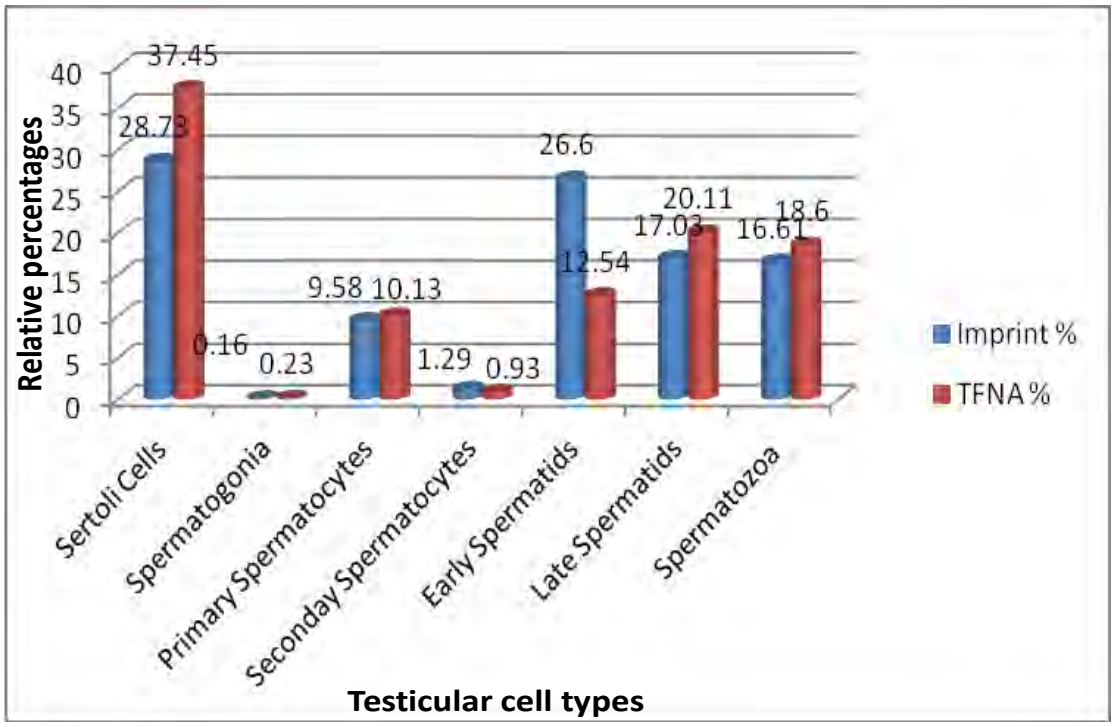


Figure 27: Relative percentage distributions of testicular Sertoli and spermatogenic cells in dromedary camel bulls during breeding seasons of imprint (blue) and TFNA (red) smears

Table 15: Testicular cell types and indexes of TFNA and imprint smears (mean ± S.E.M.) of apparently healthy dromedary camels (N=8) during the breeding season

Testicular cell types and indexes	TFNA smear		Imprint smear	
	Right Testis	Left Testis	Right Testis	Left Testis
SE	81.64±13.78	68.33±14.08	42.8±17.78	56.83±26.83
Sg	0.36±0.17	0.58±0.42	0.00±0.00	0.50±0.34
I	24.29±6.29	15.92±2.9	17.8±6.18	16.0±4.39
II	2.21±0.7	1.5±0.44	2.2±0.86	2.33±1.05
ab	24.21±4.14	26.5±4.59	46.2±13.54	47.17±13.02
cd	42.0±7.02	38.83±7.62	29.8±12.72	30.0±10.18
S	34.5±7.85	41.0±10.65	27.4±10.34	30.67±10.17
SI	21.78±4.1	24.44±5.49	25.44±6.52	23.30±2.67
SEI	134.82±56.33	263.95±139.28	33.92±13.11	82.66±51.14
SSEI	85.8±32.16	189.56±68.17	131.84±48.7	105.6±35.84

SE=Sertoli cell, Sg= spermatogonium, I= primary spermatocyte, II=secondary spermatocyte, ab=early spermtid, cd=late spermatid, S=spermatozoa, SI= spermatic index, SEI=Sertoli cell index, SSEI=spermatic to Sertoli cell indexes

In table 16, seasonal differences of testicular cytological profiles of dromedary camels for TFNA smears are presented. In most of the spermatogenic cells, Sertoli cells and cell indexes, there were significant differences between the breeding and non-breeding seasons $p<0.05$ except primary spermatocytes, early spermatids and SSEI (Spermatic index/Sertoli cell index).

Table 16: Relative numbers of testicular cells and indexes (mean±S.E.M.) in dromedary camel bulls between the two seasons

Testicular cell types and indexes	Seasons*	
	Breeding (N=8)	**Non-breeding (N=18)**
SE	75.5±9.75	46.48±2.76
Sg	0.46±0.21	2.09±0.19
I	[a]20.42±3.67	[a]17.06±1.08
II	1.88±0.42	3.89±0.61
ab	[a]25.27±3.02	[a]31.42±2.26
cd	40.54±5.07	54.63±1.89
S	37.5±6.38	71.18±3.7
SI	23.01±3.3	38.21±1.96
SEI	194.42±70.67	28.5±2.17
SSEI	[a]133.69±36.61	[a]212.44±21.84

SE=Sertoli, Sg= spermatogonium, I= primary spermatocyte, II=secondary spermatocyte, ab=early spermtid, cd=late spermatid, S=spermatozoa, SI= spermatic index, SEI=Sertoli cell index, SSEI=spermatic to Sertoli cell indexes; *Only for TFNA and columns indicated by the same letters showed values with no significant differences ($p>0.05$)

During testicular cytological analyses of dromedary camels in this study, it was also possible to demonstrate filarial worm larvae in three of the camels slaughtered during the non-breeding and one during breeding seasons. The parasites were identified in the smears of TFNA (Figure 28).

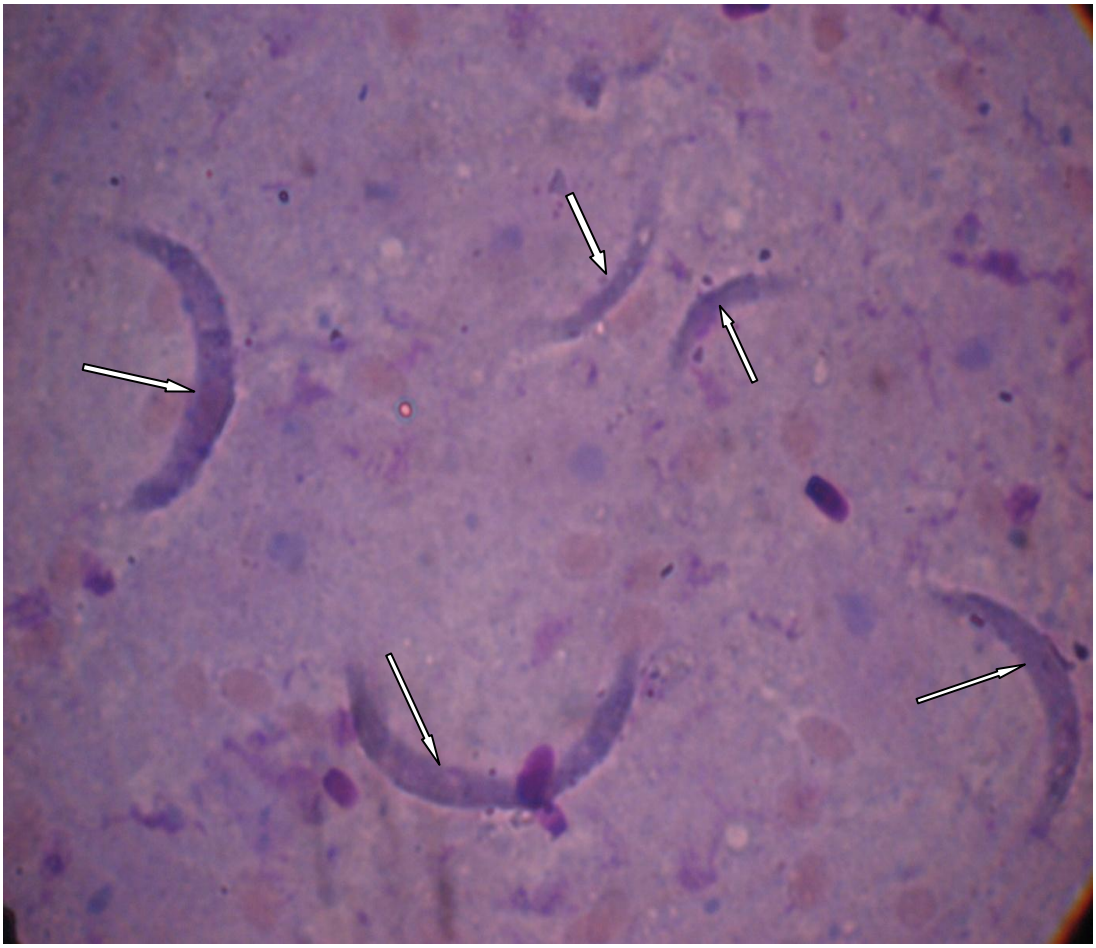


Figure 28: Filarial worm demonstrated from TFNA sample of dromedary bulls during the breeding season

5.4. Discussion

In this study, both qualitative and quantitative evaluations of testicular cytological profiles have been considered. In dromedary camels, this is the first study on testicular cytology. More importantly, the application of TFNA to evaluate cytological profiles of dromedary bulls was performed and it is found to be effective technique in this species. Using TFNA it was possible to obtain enough material from both testicles of dromedary camels for identification and quantifications of

testicular cells. It was attempted to evaluate testicular functionality in dromedary camels by quantifying Sertoli cells, spermatogenic cells, SI, SEI and SSEI using both impression and TFNA smears in breeding and non-breeding seasons of the year. Although in TFNA cytological smears normal cell-to-cell contacts are typically minimum, identification of each spermatogenic population was relatively easy as compared to imprint smears (Romagnoli *et al*., 2009).

The sizes of the testicular cells in dromedary species are smaller than those reported in different species including Iberian red deer by Romagnoli *et al*. (2009). However, the morphological characteristics and staining pattern types they take are similar to all reports from previous reports in different species (Romagnoli *et al*., 2009; Santos *et al*., 2010; Stelletta *et al*., 2011). The morphology of testicular cells in dromedary camels is similar to alpaca testicular cell morphologies as described by Stelletta *et al*. (2011) and it is not surprising since they have evolved from same ancestor.

The percentage cytological profiles in dromedary camels during the non-breeding season appears in the decreasing order from spermatozoa, early spermatids, late spermatids, Sertoli cells, primary spermatocytes, secondary spermatocytes and finally spermatogonia. However, in the breeding season the relative percentages showed highest number of Sertoli cells followed by early spermatids, late spematids, spermatozoa, primary spearmatocytes, secondary spematocytes and spermatogonia. These proportions more or less showed normal evolutionary steps of spermatogensis from spermatogonia to spermatozoa and comparable to a report by Stelletta *et al*. (2011). However, it is possible to appreciate in this study that Sertoli cell number is maximum during the breeding season as it signifies their anatomical and physiological support to the spermatogenic cell development during the rutting period than non-breeding season.

The main intension of this study was to determine the application of TFNA in *Camelus dromedarius* in comparison to impression smear as histological counterpart with the ultimate goal of TFNA application in evaluation of spermatogenesis in dromedary bulls. Histological approaches were taken as gold standard and since recently comparison of TFNA with impression smear have been studied in human and different animal species (Abdelmoneim *et al*., 2004; Stelletta *et al*., 2011). These previous studies confirmed and suggested application of TFNA in their respective studies. In this study, it was found that there is correlation between the two smears cytological profiles of dromedary camels in the rutting season. However, some differences were seen in the cytological profiles and indexes during the non-breeding season between the two smears.

The differences do not signify that TFNA's failure as we have drawn enough testicular cells and in most cases even more cells count than imprint smears. There are also some statistically significant differences between left and right testes during the non-breeding season of dromedary camels in contrary to the report by Stelletta *et al*. (2011) in alpacas. The probable reason might be attributed to the differences in testicular size and activity between right and left testicles in dromedary bulls as left testis is always larger in size than the right testis. However, no significant differences were observed between the two sides during the rutting/breeding season.

The seasonal comparison among the numbers of different spermatogenic cells, indexes and Sertoli cells showed significant differences in most of the cases of this study. The numbers of testicular cells and indexes differences between breeding and non breeding seasons are expected facts. However, it is difficult to interpret how the testicular cytological profiles were higher in non-breeding season than the breeding season. The most probable reason might be due to technical errors during counting, staining or sampling processes. Otherwise, it is not possible to interpret the physiological facts whereby cellularity is more in non breeding season than rutting

season unless some pathological situations existed. Animals outside the breeding season have higher SI, they were not ejaculating so there was an accumulation of sperms in the testis.

The SEI demonstrated in this study showed significantly higher value in the breeding season due to the fact that higher number of Sertoli cells count in rutting period. On the other side the higher SEI in breeding season is due to the lower concentration of sperm, though no significant difference in SEI, which means all animals have the same sperm productivity potential.

It was difficult to compare our findings with previous reports of dromedary camel testicular cytological profiles due to absence of published literature. However, both qualitative and quantitative analyses in different animal species and humans were considered for comparison and interpretations. Most of the results are comparable to report by Stelletta *et al*. (2011) in alpaca since they are from same family though some differences were observed.

While studying the testicular cytological profiles, we accidentally diagnose some filarial larvae in four dromedary bulls slaughtered during the study period all from TFNA smears. Therefore, this study agrees with others that TFNA can serve as a minimally invasive technique in the diagnosis of infection causing epididymitis and epididymo-orchitis (Arora *et al*., 1996; Reddy *et al*., 2004; Sah *et al*., 2006). A study by Mitra *et al.* (2009) described aspirates from 4 scrotal swellings which showed numerous coiled and uncoiled sheathed microfilariae along with neutrophils, eosinophils and few lymphocytes. Thus aspirates from epididymal nodules of microfilarial infection can yield microfilaria with variable amount of inflammation, and the cytopathologist should screen all aspirates from epididymal nodules with eosinophils for the presence of this parasite.

The possible disadvantages of TFNA as diagnostic technique include the aspiration technique requires practice and skill, some percentage of the aspirates are unsatisfactory, interpretation requires experience and diagnostic material is mostly limited. Different previous reports suggest the interpretation can be much better if one personnel takes care of all steps.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

Camels are important livestock species for the livelihood of the Afar pastoralists where other animals are of less adaptive and productive. Reproductive performance parameters of the Afar camels are found to be almost comparable to those of the Kenyan breeds. Generally, the present study shows that the productivity of the Afar camel is very much lower than it should be in terms of number of calves delivered in her life time and needs well studied management interventions. Under proper management intervention (feeding, health care, mineral supplementation and keeping proper bull to female camels' ratio) in to the reproductive parameters, it is possible to increase the performances of the camels. Pastoral areas have huge potential of research in the livestock sector in general and camels in particular as the unforeseen desertification widens. Researchers and funding agencies should give priority to such areas of research in order to solve the problems of livestock sectors. There has to be extensive study to evaluate the reproductive and production performances of camel breeds of the country, so that the outcomes will suggest which breeds should be kept for what purposes as to the demand of the society and for the national economy. Breeding management should be improved and proper records should be kept of births, mating and possibly of production and awareness creation among pastoralists.

Traditional nomadic, is the main type of management system practiced by Somali pastoralists of the study areas. Camel, cattle, goat and sheep were the main types of livestock reared in the area with high preference of camels over other livestock. She camels were dominant in the herd followed by female calves. Participation of hired labourers in camel management is increasing from time to time in the study areas and this might favour for the children of the pastoralists opt for education. Disease, feed shortage and security were the main constraints of camel production in Somali

pastoralists. Camel pox, anthrax, trypanosomiasis and respiratory diseases were the most prevalent diseases in the study districts of Somali pastoralists. Cactus and predators were also the emerging challenges for camel owners in the study areas as a consequent of drought. Female camels were very rarely culled due to diseases, old age and poor production and reproductive performances. Inbreeding might be an unnoticed problem for the camel owners of the areas as they use only one breeding male for several years with same pedigree to specific camel herd. Generally, the reproductive performances of the Somali camel breeds seem to be lower. High abortion rate was recorded and more calves died before reaching weaning age. Extensive study to evaluate the reproductive and production performances of camel breeds of the country should be conducted to select the most productive breeds for specific purposes. Regular training for real pastoralists and development agents on proper management of calves, lactating and pregnant camels, disease prevention, control, surveillance and reporting system to the respective bodies should be devised. Among the study districts of the three regions, it seems that Somali and Afar have more likely hood to be dependent on camels than Borana pastoralists. In Borana, some pastoralists grow crops in addition to animal husbandry though majority of them are dependent on cattle and camels for their livelihood.

Macroscopically different pathological abnormalities of reproductive organs of female camels were recorded and affected organs were processed in the laboratory for histopathological characterization and bacterial isolation. Endometritis and ovarian cysts were the major reproductive organ problems recorded during the study period. About seven types of bacterial genera were identified from endometritis cases, in which *E.coli* and *staphylococcus* species were the major bacterial isolates identified in this study. The findings suggest frequent detection of the clinical diseases of female camel's reproductive organ to address the corrective, control and prevention methods for improving of the reproductive performance of this species. The role of each reproductive problem incriminated as causes of reproductive failures (infertility) in this species of animal needs further investigation and the socioeconomic role of these problems in pastoral areas of the country needs to be

specified. Further study of causes of reproductive organs disease and disorders, isolation and identifications of viruses and fungi, immunohistochemistry and molecular techniques for specific organism in lesions with causal relationship should be conducted. Collaborative research activities should be practiced to understand the productivity of *Camelus dromedarius* and devise proper intervention mechanisms.

Viral diseases are circulating among Ethiopian camels and they may play their own negative roles to the productivity by affecting reproduction. Among all the viruses, blue tongue virus has the biggest sero positivity. However, Akabani, Chuzan and Japanese encephalitis viruses were also with higher sero positivity among Ethiopian dromedary camels.

The current study confirms the application of TFNA as diagnostic tool for breeding soundness test of dromedary bulls. The results also signified that, this technique is applicable to diagnose pathological conditions in this species without any surgical procedures. The staining characteristics and morphological structures of testicular cells in dromedary bulls are more or less close to other domestic species. Testicular cytological profiles of dromedary camels vary significantly between rutting and non-rutting periods.

Under proper management intervention (feeding, health care, mineral supplementation and keeping proper bull to female camels' ratio) in to the reproductive parameters, it is possible to increase the performances of the camels. Pastoral areas have huge potential of research in the livestock sector in general and camels in particular as the unforeseen desertification widens. Researchers and funding agencies should give priority to such areas of research in order to solve the problems of livestock sectors. There has to be extensive study to evaluate the reproductive and production performances of camel breeds of the country, so that the outcomes will suggest which breeds should be kept for what purposes as to the demand of the society and for the national economy. Breeding management should be improved and proper records should be kept of births, mating and possibly of

production and awareness creation among pastoralists. Best practices from more experienced camel keepers and regions should be scaled up and use extensively to other areas. Use of single bull for several she camels for long time may bring about inbreeding and dissemination of infection. Therefore, proper follow up and selection of bulls for proper number and pedigree she camels should be practiced for productivity in this species.

The role of each reproductive problem incriminated as causes of reproductive among dromedary camels in Ethiopia needs further investigation. The socioeconomic effects of these reproductive tract problems in the pastoral areas need to be specified. Collaborative researches are critically important to improve the productivity of *Camelus dromedarius* and devise proper intervention mechanisms for the identified problems. To recognize the ecology of these viruses and control camel diseases in Ethiopia, further large scale epidemiological investigations is required. Optimization of the technique in terms of sampling site, needle and syringe size, age of animals, seasonal differences, and personnel experiences factors is however recommended for wider application in dromedary camels.

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APPENDIXES

Appendix 1: Questionnaires to camel breeders in Ethiopia

1. General household information
Owner's name: .....
Village: .....
Owner's Number: .....
Level of education: .....
Age: .....
Sex: .....
1.1- Labor distribution in camel production

	Dairy production					
	Feeding	Milking	Breeding	Herding	Health care	Housing
Husband						
Wife						
Sons						
Daughters						
Laborers						

1.2- What types and number of livestock do you keep?
a) Camel _____b) Cattle _____ c) Sheep: _____ d) Goats _____.e) other _____
1.3- If you have camels, cattle, sheep and goats, could you rank them according to the relative importance to you?
a) Camel _____ b) cattle _____ c) sheep _____ d) goats _____
1.4- How is composition of your herd?
a) Number of she camel _____ b) Number of she camel U. In. _____ c) Number of camel _____ d) Number of female calves _____ e) Number of castrated camel ____ f) Number of male calves __

2- Herd management

2.1- What is type of your management system?
a) Traditional nomadic _____ b) transhumant _____ c) sedentary ___
2.2- Did you migrate or move with animal during year? a) Yes b) No
2.3- If yes: where did you move during a) wet season _____ b) Dry season ____

3- Farming system:

3.1- Did you grow crops? Yes No
3.1.1- If yes: Did you sell any crops during the past 12 months? Yes No
3.1.2- If yes which crop did you sell?
3.2- What do you consider your main production activity?
a- livestock _____ b- farming _____ c- livestock and farming _____

4- Breeding practices

4.1- Do you keep a breeding camel? YES ____ NO ____

4.1.1- If YES: Why do you keep a camel (s)? _____

4.1.2- How many breeding camels do you have? _____ What is the breed and age of camel (s) you are owning?

No.	Breed	Age
1		
2		
3		
4		

4.1.3 If NO: Why do you not have a breeding camel? _____ _____. (*and go on to question no. 5.6)*

4.2- Where is your breeding camel from?

a) Own herd ___ b) other herd ___ c) purchased ___ d) other ___

4.2.1- If (a) own herd: At what age do you select your breeding camel? ____years ____months

4.3- What do you do with camels that are not selected for breeding purposes?

a) Castrate ___ b) just leave them in the herd ___ c) sell (before mature) ____ d) other ____

4.4- Do you select your own camel? YES ___ NO ____

4.4.1- If YES: How do you choose a breeding camel, what are the characteristics you use to select your breeding camel?

a) _____ b) _____ c) _____

d) _____ e) _____

4.5- How long do you keep a breeding camel for service? ____years

4.6- Where do you take the replacement breeding camel from?

a) Own herd ___ b) other herd ___ c) purchased ___ d) other ___

4.7- Can the replacement camel be the son of the former breeding camel? YES ___ NO ___

4.7.1- If NO: Why not? _____

4.8- How do you make sure that your breeding camel is fathering the herd? ___ _____

5- Mating organization:

5.1- Do you keep mating records of your camel (s)? If yes how? _____ _____

5.2- What are the mating records you keep (observation of the records)? _____ _____

6- Production and reproduction performance:

6.1- What was the average quantity of milk you got from yours she-camel last time and how long did you milk your she camel?

She camel No.	Daily milk yield (l)			Lactation length (months)
	Beginning of lactation	Middle of lactation	End of lactation	
1				
2				
3				
4				

6.2- What was the age of your she-camel when they gave birth to their first calf?

She camel No.	Birth date	First calving date
1		
2		
3		
4		

6.3- When did your She camel give their last calving and previous calving?

She camel No.	Last calving date	Previous calving date
1		
2		
3		
4		

6.4- How many times have you taken yours she-camels for camel before they get pregnant last time?

She camel No.	Number of services
1	
2	
3	
4	

7- Production objectives:

7.1Why do you keep camel?_____(first reply given)

7.2- From the following list, could you rank the reasons according to the degree of importance**?**

Reasons	Rank
Income from sale of milk	
Milk for home-consumption	
Income from sale of animal	
Traction (animal for work)	
Manure	
Insurance against financial problems	
Investment (Like a bank)	

8- Feeding Management, Animal health and Production Constrains:

8.1.1- What do you feed your animals?

a) Grazing _____ b) hay _____ c) crop residues _____ d) Concentrates _____ e) minerals _____

8.1.1.1- If you use hay, which animals do you supplement with it? _____

8.1.1.2- If you use concentrates, which animals do you supplement with it? _____

8.1.2- Do you consider that the feeding is a constraint to your herd production?

8.1.3- Do you consider that the water supply is a constraint to your herd production?

8.1.4- How did you secure water supply to your camels? In wet season, Free_____ Paid _____

8.1.5- How did you secure water supply to your camels? In dry season, Free_____ Paid ____

8.2.1- What are the prevalent diseases in your area?

a) _____ b) _____ c) _____ d) _____

e) _____ f) _____

8.2.2- What is the most important one? _____

8.2.3- Did you report any diseases among your herd during past 12 months? YES ____NO ____

8.2.3.1- If YES: could you mention them?

a) _____ b) _____ c) _____ d) _____

e) _____ f) _____

8.2.4- If you report any case of disease, where you look for veterinary help from?

a) Government veterinary service _____ b) private veterinarians _____

c) Drugs suppliers _____ d) others _____

8.3- Could you rank these below constrains according to relative importance?

a) Lack of pasture _____ b) security ___ c) lack of water ___ d) diseases _____

e) Capital _____ f) labor _____

8.4- What do you consider a more serious constraint to your camel production?

9- Do u sell female camels? Yes _____, No_____

If yes what are the reasons to sell them? _____

Appendix 2: Progeny history questionnaire to determine reproductive performances

First the names of all breeding females in the herd have to be listed.
Thereafter, the enumerator chooses one breeding female at a time from the list and asks the following questions for each and every one of them.

A. Questions concerning the breeding female:

1. Name of the breeding female
2. Was she born in your herd or did you get her from somewhere else?
3. If born in herd, when was she born? (*Give date as "year/season")*
4. If acquired, when did you get her? (*Give date as "year/season"*)

   - ✓ Did you buy her; get her as gift or as dowry?
   - ✓ Was she a calf, heifer or adult at that time?
   - ✓ How old was she at that time?
   - ✓ Why did you get/buy her?

5. What breed is this dam?
6. Is this dam a good, average or poor milker?
7. Does she have any milking problems like udder abnormalities, mastitis, poor milk let-down or else?
8. How many calves did this dam deliver up to now? (*The complete number, alive as well as dead ones*)
9. Did this dam have any abortions?
   - ✓ If yes, were those early or late abortions?
10. Did this dam ever show difficulties to conceive?
    - ✓ If yes, what do you think was the reason?
11. Is the dam now pregnant (and since when), or is she lactating (and since when)?

B. Questions concerning the calves (to be asked for all calves, dead and alive ones, starting from the first)

1. When was the calf born? (*Give the date as "year/season"*)
2. Which was the sex of the calf?
3. Did the calf survive until weaning?
   - ✓ If no, when did it die? (*Give date as "year/season"*) or how old was it when it died?
   - ✓ If no, what was the cause of its death?
4. Where is the calf now? (In your herd, sold, slaughtered, given away, died after weaning)
   - ✓ If disposed, when was it disposed? (*Give date as "year/season"*) and why did you dispose it off?
   - ✓ If dead, when did it die (*give date as "year/season"*) and what did it die of?

CURRICULUM VITAE

Personal Information

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Work experience	2007-2009 at Dilla University
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Education

Since October 2009	Addis Ababa University, College of Veterinary Medicine and Agriculture, PhD student
September 2005- August 2007	Addis Ababa University, Faculty of Veterinary Medicine: MSc
September 1999- July 2005	Addis Ababa University, Faculty of Veterinary Medicine: DVM

Publications

1. **Simenew, K.,** Fekadu R., Tesfaye S. T., Tesfu K., Juri V., David O. O., Calogero S. (2014). Testicular cytological profiles of apparently healthy dromedary camel (*Camelus dromedarius*) bulls *(Submitted to Journal of Animal Reproduction Science).*
2. **Simenew, K,** Moa, M., Ashenafi, F., Tilaye, D., Fekadu, R., Getnet, M., Gizat, A., Tesfaye, S. T., Tesfu, K., Fufa, D. (2014). An abattoir survey of lesions of the internal reproductive organs of female dromedary camels in Ethiopia (*Submitted to BMC Veterinary Research)*.
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23. Getachew, A., **Simenew, K.** (2012). Entrepreneurship Practices among TVET Graduates, LAP LAMBERT Academic Publishing, Germany, ISBN: 978-3-8473-4186-4, 102p

24. Simenew, K., Gezahegne, M., Wondefraw, M., Getachew, M., Lemma, A., Eyob, I. (2011). Reference values of clinically important physiological, hematological and serum biochemical parameters of apparently healthy working equids of Ethiopia, *Global Veterinaria,* 7(1):1-6, 2011.

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1. Since July 22/2007 - Instructor and Faculty Dean of Applied Agriculture and Rural Development, Dilla University, Dilla, Ethiopia
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3. Member of Senate Legislation Development Committee of the University
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5. Member of the University management
6. Member of the University senate

7. Member of the Strategic Plan Development Committee of the University
8. Member of steering committee of BPR of the university and participate in different Academic and Administrative activities

Employer	Dilla University
Academic position	Assistant Professor
Computer Skill	Basic computer skills: Microsoft Office, SPSS, MS Project, SAS
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Organizational skills and competence	Coordination and administration of people, projects and budgets: acquired at Dilla University.
Technical skills	Working on and handling animals.
English Language Proficiency	Excellent in reading, writing and verbal skills

SCHOLARSHIP AWARDS, INTERNATIONAL CONFERENCES AND WORKSHOPS:

1. Training scholarship on Infectious diseases of Zoonotic importance at Nihon University, Japan for young researchers in March /2013.
2. Researcher visit to Nihon University through RRIAP Japan, August/2013
3. Coimbra group Universities Scholarship at Padova University for young researchers for Sub-Saharan countries from October to December/2013.
4. ARISE-INTRA-ACP scholarship at Makerere University funded by European Commission February 2015.
5. TRAINING COURSE "Dromedary Camel Health and Diseases and Veterinary Cooperation in Developing Countries" 4-6, Nov 2013, Section of Veterinary Clinics and Animal Production (DETO) Valenzano (BA), Italy
6. Training workshop on reproductive biotechnology of dairy sheep Enna, Cecilia, Italy, December, 2013.
7. Africa BOMA Culture international workshop on "one health", Kampala Uganda, August, 2014.

PROFESSIONAL AFFLIATIONS AND MEMBERSHIP

-Member of "The Vet 2011 Animation and Coordination Committee" and Ambassador of this project in my Country
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