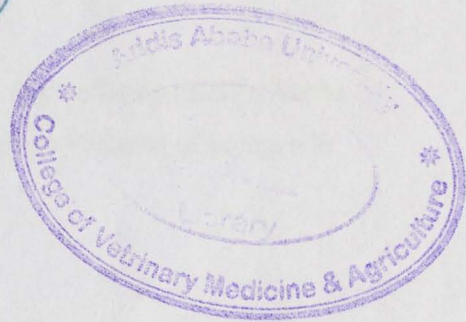
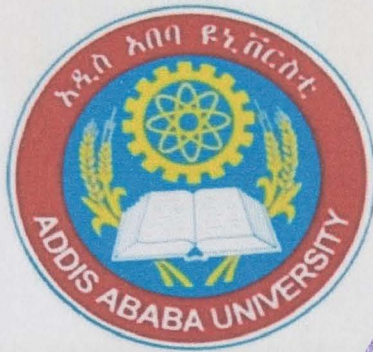


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**PATHOLOGICAL AND BACTERIOLOGICAL STUDY ON ABNORMALITIES OF
FEMALE REPRODUCTIVE ORGAN OF *CAMELUS DROMEDARY* SLAUGHTERED
AT AKAKI ABATTOIR, ADDIS ABABA**

Msc Thesis



BY

Moa Melaku

**Addis Ababa University College of Veterinary Medicine and Agriculture, Department of
Pathology and Parasitology**

JUNE, 2014

BISHOFTU, ETHIOPIA

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FEMALE REPRODUCTIVE ORGAN OF *CAMELUS DROMEDARY* SLAUGHTERED
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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical veterinary pathology

By

Moa Melaku

June, 2014

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Pathology and Parasitology

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Moa Melaku** entitled: **Pathological and Bacteriological Study on Abnormalities of Female Reproductive Organ of *Camelus Dromedary* Slaughtered at Akaki Abattoir, Addis Ababa** and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Tropical Veterinary Pathology.

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DEDICATION

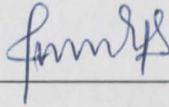
This thesis manuscript is dedicated to my father, Melaku Shigut, and my mother, Belayinesh leta, who she passed away last year, for nursing me with affection and love and for their dedicated partnership in the success of my life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my original work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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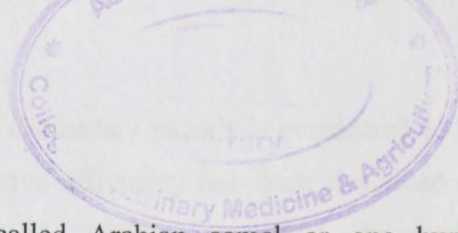
ABBREVIATIONS

COD	Cystic Ovarian Disease
CL	Corpus Luteum
EMB	Eosine Methylene Blue
GnRH	Gonadotropin Releasing Hormone
HCG	Human Chorionic Gonadotropin
HE	Haematoxyline Eosin
IMViC	Indole Methyle red Vogos Prouskur Citrate
LC	Luteal Cyst
MR	Methyle Red
NAHDIC	National Animal Health Diagnostic and Investigation Centre
OF	Oxidation Fermentation test
PMNs	Polymorpho Nuclear cells
PGF ₂ α	Prostaglandin
SCN	Supra Chiasmatic Nucleus
VP	Vogous Proskuer
XLD	Xylose -Lysine Desoxycholate

ABSTRACT

A cross-sectional study was conducted on reproductive organs of Camelus dromedarius to characterize the pathological lesions and to isolate and identify possible bacterial causes of reproductive failures. Reproductive organs of 231 female dromedary camels (169 from Borana and 62 from Afar) were grossly inspected and sampled for further histopathological and bacteriological studies. Grossly 46(19.9%) camels were found with different types of reproductive organ disorders, (30 (17.8%) from Borana and 16 (25.8%) from afar). Ovarian 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%), were the identified disorders. The frequency of lesion occurrence did not show statistically significant difference ($P>0.05$) at different age groups and areas of origin; whereas the occurrence of reproductive tract abnormalities were significantly different ($p<0.05$) between pregnant and non-pregnant camels with higher occurrence in non pregnant animals. Uterine abnormalities were the major disorders recorded in this study accounting for 31 (13.4%). From 31 cases with endometritis 26 (83%) were positive for different types of bacterial cultures. Escherichia coli (38.7%) Staphylococcus spp (35.5%), Streptococcus spp (25%), Enteriobacter aeruginosa (16.1%), Corynebacterium spp (12.7%), Proteous spp (9.7%), and Salmonella spp (6.5%) are bacteria isolates. In conclusion the finding of the present study suggests frequent detection of the diseases of dromedary female camels' reproductive organs. Considering the importance of Camelus dromedarius among the pastoral society of Ethiopia, there should be collaborative studies pertinent to reproductive health for the betterment of production and productivity of camels.

Key words: Bacteriology, Camelus Dromedarius, Endometritis, Pathology, Reproductive organ



1. INTRODUCTION

Camelus dromedaries also called Arabian camel or one humped camel, was first domesticated 5,000 years ago in the Arabian Peninsula (Ouajd and Kamel, 2009). Camel is an important species uniquely adapted to hot and arid environments, and as such contributes significantly to the food security of nomadic pastoral households. Camels are hardier, because they graze on shrubs and trees that other livestock do not (El-Bahrawy, 2012).

In Ethiopia, they are the subset of huge livestock resource when considered from daily livelihood for millions of pastoralists and national economic point of view (Getahun and Kassa, 2000). They play an important socio-economic role in Ethiopia, where nomadic and semi-nomadic pastoral and agro-pastoral production systems predominate. Camel is the major source of the highly valued milk supply and of transport energy in addition to its considerable contribution to meat supply for the human population in and out of their area of production. Moreover, the role of camel as cash income generator at producer as well at national levels is highly esteemed. Camel serves as an indicator of wealth and high social status, particularly in the society of pastoralists and agro-pastoralists of eastern and southeastern Ethiopia (Hailemariam *et al.*, 2008).

Camels have been endowed with particular anatomical and physiological traits, and a relatively low susceptibility to a wide range of clinical diseases. These have provided them with endurance for survival and to undertake difficult tasks (Hassan, 2007).

The reproductive system is not essential to the survival of an individual animal but is essential to the survival of a species and perpetuation of generation (Maxie, 2007). Short breeding season is an important factor for the low reproductive performance of dromedary camel. It remains as a major obstacle to the growth of population of dromedaries. Male and female camels are both seasonal breeders. They breed only during certain times of the year (Babiker *et al.*, 2011).

The reproductive efficiency of dromedary camels is considered to be low (Abdel-Aziz and Nasser, 2013). Poor reproductive efficiency has been described as a major problem in camelids (Tibary and Anouassi, 2001). Anatomical abnormalities and pathological processes of female reproductive tract have been reported as the main causes of infertility (Deen, 2013). Infectious diseases of the reproductive system of camels may cause complications resulting in infertility or poor reproductive performance and consequent loss of productivity (Adel *et al.*, 2012). Thus, identification of reproductive diseases is important, especially when dealing with genetically superior animals (Ali *et al.*, 2010).

In Ethiopia until recently there has been very little systematic research on camels' reproductive disorders (Bekele, 2004). Despite the benefits of camels regarding their meat and milk, camel production is still not undertaken on a commercial scale (Abdel-Aziz and Nasser, 2013). The exploitation of productive capability in camel is only possible if the reproductive potential can be improved (Srikandakumar *et al.*, 2001). As camels are generally used in less well-developed countries, researches into improving fertility have been lacking (Skidmore, 2005). For the diagnosis and management of reproductive performance in camels as well to circumvent the reproductive disorders, isolation of bacteria from uterus and histopathological investigations are critical (Mshelia *et al.*, 2012). Likely in Ethiopia there is a gap in identifying the reproductive problem related with infertility. Thus the present study is attempted with the major objectives:

- Characterizing the pathological disorders and lesion of reproductive organs of camels.
- Isolating and identifying bacteria from lesion of the reproductive organs with gross abnormalities.

2. LITERATURE REVIEW

2.1. Reproductive Efficiency and Physiology of Camels

The reproductive efficiency of camels under natural conditions is generally regarded to be low. The reasons for this low reproductive efficiency of the camel (*Camelus dromedarius*) is probably due to the relatively short breeding season, a longer prepubertal period, a long gestation period of 13 months, a prolonged (8–10 months) period of lactation-related anestrus leading to a long inter-calving interval, limited oestrus period, poor pastoral management systems, inadequate nutrition and the lack of use of assisted reproductive techniques such as embryo transfer and artificial insemination (Al-Juboori and Baker, 2012).

However rainfall and subsequent availability of improved nutrition was the main trigger for camel sexual receptivity. The ovarian activity changes show highly significant differences between months, as well as, between seasons (Marai *et al.*, 2009). The she-camel, although it shows strong tendency to be regarded as a seasonal breeder, pregnancy can occur at any season of the year as a polyestrous animal. However, in all cases, sexual activity of the females coincides with that of the males and both respond to the same environmental conditions (Ouajd and Kamel 2009). Globally, the breeding season of the camels begins at different dates beginning of September and ends at different dates until June in the different parts of the Northern World and from June to September in the Southern parts of the World, which are the mildest periods of the year, but with decreasing and / or increasing daylight, while the non-breeding season is in summer hot months. The severe hot conditions (which are strongly related to the length increase of the photoperiod) under which the camel lives directly without any shelter in summer (usually) disturb the physiological functions that affect deleteriously the sexual activity and all the related traits of the camels' polyestrous nature. The onset of the breeding season is triggered by availability of fresh green feed. Thus the breeding season coincides with the rainy period of the year (Yegil, 2001).

However, although photoperiodic variations have a strong influence, yet there is some evidence suggesting that the suprachiasmatic nucleus (SCN) may be sensitive to changes in ambient temperature, with some cells being more responsive to cold and others more responsive to heat. Furthermore, the molecular mechanisms that regulate rhythmicity, such as the cyclic changes in the expression of clock proteins, can be altered by temperature changes. Further studies on the importance of SCN in reproductive functions of the camel, are needed. SCN is an important structure regulating circadian and seasonal rhythms of most biological functions in mammals, particularly reproductive function and behavior, including the phasic and tonic release of hormones (Marai *et al.*, 2009).

2.2. Camel Rearing Areas in Ethiopia

Camels inhabit almost all peripheral drier lowlands that generally fall below 1,500 meters above sea level in Ethiopia, except the western areas where there is high humidity. These areas include the major parts of the Somali and Afar National Regional States and some parts of the Oromia National Regional State. Afar's camels are small but extremely hardy and, except for some used for salt transport, are kept almost entirely as milk producers. Ethiopia's largest camels are of the Ogaden type, owned by the Somali in the east of the country. Borana camels (found in the Oromia National Regional State) are of intermediate size and are employed as pack, draught and dairy animals. The eastern part of Ethiopia is considered the heartland of the camel population, because it is home to two-thirds of the nation's camels. Although the contribution of camels to the national economy is not fully quantified, estimated that 20,000 tones of camel meat and 174,000 metric tones of camel milk can be produced annually in the country (Tezera and Kassa, 2012).

In Ethiopia camels are kept in the arid and semiarid lowlands of Borana, Somali and Afar regions, which cover large parts of the pastoralist areas in the country (Woubit, *et al.*, 2001). All camels in Ethiopia are owned by pastoralists and agro pastoralist inhabiting almost all peripheral drier lowlands of Ethiopia that generally fall below 1,500 meters above sea level, except the western areas where there is high humidity (Dawo, 2010)

The eastern part of Ethiopia is considered the heartland of the camel population, because it is home to two-thirds of the nation's camels. The Borena rangeland of southern Ethiopia is the third important camel production region of the country, the first and second being Somale and Afar regions. The Borena pastoralists probably started camel production in early 1560 in the *Gedda* period of *Abbay Orro*. It has been also speculated that the Muslim Gebra communities were said to be instrumental in introducing camels to the Borena plateau. Thereafter, social and changes, extensive seasonal migration and change in rangeland ecosystem (decreasing pasture for grazers and increasing browse vegetation species) are major factors for the expansion of dromedaries into the area. Afar's camels are small but extremely hardy and, except for some used for salt transport, are kept almost entirely as milk producers. Ethiopia's largest camels are of the Ogaden type, owned by the Somali in the east of the country. Borena camels (found in the Oromia National Regional State) are of intermediate size and are employed as pack, draught and dairy animals (Bekele, 2004).

2.3. Abnormalities of Reproductive Organs of Female Camel

2.3.1. Diseases of ovary

Ovarian pathology is responsible for a great number of long standing infertility problems in the dromedary camels (Tibary and Anouassi 2000). Pathological examinations of ovaries provide definite information more accurately than that obtained by clinical or other paraclinical examinations due to its anatomical location and diagnostic techniques limitations (Nourani and Khodakaram, 2004). The most commonly detected abnormalities of ovaries among dromedary camels are discussed.

2.3.1.1. Ovarian hypoplasia (inactivity)

The sizes of the ovaries are determined by the age, size, health and stage of female reproductive cycle (Yagil, 2001). Absence of ovarian follicular activity is a frequent

condition in *Camelidae* due to congenital or acquired ovarian hypoplasia. Ovarian activity is greatly affected by body condition, lactation, Reproductive status, physiological state disease conditions and use of the animal. Ovarian activity is reduced in camels just retired from racing and in camels with a low body condition score (< 3). Ovarian hypoplasia due to genital or chromosomal abnormalities is also possible. Clinical diagnosis of ovarian inactivity or hypogonadism is based on findings at rectal palpation, ultrasonography and endocrine profiles. From endocrine point of view ovarian hypoplasia should be suspected if estradiol levels remain very low (Tibary and Anouassi 2000).

The affected ovaries on both sides are small (half to one third), smooth, firm, with no palpable follicles (very small follicles) or corpus luteum, showing no evidence of ovulation and the uterus is small, atonic (Tibary and Anouassi, 2000; Ali *et al.*, 2010). Microscopically hypoplastic ovary is characterized by atrophy of the ovarian cortex, which consisted almost exclusively of fibrovascular connective tissue (excessive fibrous connective tissue proliferation), with no evidence of follicular activity (complete absence of follicle or luteal developments) (Shawky *et al.*, 2004; Adel *et al.*, 2012).

2.3.1.2. Ovarian cysts

Cystic ovarian disease (COD) is an important ovarian dysfunction and a major cause of reproductive failure. It is generally accepted that disruption of the hypothalamo-pituitary-gonadal axis by endogenous and/or exogenous factors, causes cyst formation. COD prolongs the calving interval, loss of production and reduces reproductive performances ultimately (Mahmoud *et al.*, 2011).

Taking the place of occurrence in to account, Cystic conditions of the ovaries are of two types: cysts within the ovary itself (ovarian cysts) such as follicular or luteal cysts and cysts outside of the ovarian tissue known as para-ovarian cysts. The term "cystic ovaries" does not always apply to *Camelidae* due to the fact that a large proportion (30 to 40%) of females

develop some form of follicular cyst if not bred given that ovulation in these species is induced (Tibary and Anouassi 2000).

Ovarian cysts are described according to the structure involved and their appearance and classified as follicular cysts, luteal cysts, cystic corpora lutea or hemorrhagic cysts according to their histological and physical characteristics. Follicular and hemorrhagic cysts are normal evolution of the non ovulatory follicle in 30 to 40% of females. Cystic conditions of the ovaries are easily diagnosed by ultrasonography. They can be single or multiple and can be as large as 12 cm in diameter and can weigh as much as 250 g (Tibary and Anouassi 2000).

Follicular cysts:

Follicular cysts are unovulatory follicles that persist for a minimum of 10 days, have a diameter greater than 2.5 cm and are characterized by either continuous estrus or anestrus (Johnson, 2008). Cystic ovarian disease results from a neuroendocrine imbalance (dysfunction) involving the hypothalamic-hypophyseal-gonadal-axis (hypothalamic-pituitary-ovarian axis) resulting in ovulation failure. The development of follicular cysts is because of the compromised positive feedback of estradiol on release of gonadotropin-releasing hormone (GnRH). Follicular cysts were either solitary or multiple, with average diameter 3–5 cm. Large cysts exceeding 5 cm in diameter can also be seen, in which case the rest of the ovary is atrophic. Cysts contain a clear, yellowish fluid, and a few filled with sanguineous fluid (Adel *et al.*, 2012). Their walls are thin, tense, transparent or semitransparent and have clear congested blood capillaries on their surfaces, especially in cysts of large diameter. Meanwhile, the cysts of small size, less than 1.5 cm in diameter, have partially opaque, thick white fibrous walls. The cut section of the thin walled cysts revealed yellowish serous fluid, while those of thick wall showed viscous or gelatinous yellow substance (Shawky *et al.*, 2004).

Microscopic examination showed the cysts to be lined with one or more layers of granulosa cells and the endometrium are edematous while the uterine glands were hyperplastic (Adel *et al.*, 2012). Most of these cysts have thick vascular connective tissues wall and lined by many layer of granulosa cells. Moreover, some of these cysts especially larger ones resulted in pressure atrophy of the adjacent ovarian tissues and bring about atrophication of the whole ovarian mass (Shawky *et al.*, 2004).

Luteal cysts:

Luteal cysts develop when ovulation fails to occur and the theca undergoes luteinization. Luteal cyts (LC) are considered to be the later form of ovarian follicular cysts and therefore the causes to follicular cysts can also be considered the original causes of luteal cysts. The luteal cyst occurs when the cells of the follicular cyst (granulosa and theca) become luteinized and start producing progesterone (Mahmoud *et al.*, 2011). Luteal cysts are usually single, thick-walled, grayish-yellow and smaller in size than follicular cysts. Persistent corpora lutea are rare in the female *Camelidae*. However, the condition has been suspected on the basis of prolonged elevated plasma progesterone levels in the absence of pregnancy.

The persistence of luteal function (high progesterone concentrations) is rarely due to maintenance of the corpus luteum but rather to the luteinization of hemorrhagic follicles (Tibary and Anouassi 2000). Luteal cysts appeared as dark red masses protruding from the ovarian surface and filled with a colorless, semi-coagulated secretion. The lumen of the cysts is surrounded by capillary-rich connective tissue and a combination of normal, degenerated, and vacuolated luteal cells (Adel *et al.*, 2012).

Microscopically, the wall of cysts formed from small theca-luteal cells and fat containing granulosa cells. They contained homogenous eosinophilic structure less substance mixed with few luteal cells in the lumens. Moreover, pressure atrophy of the ovarian structure may also follow (Shawky *et al.*, 2004).

Hemorrhagic cysts:

The cysts have a thick and highly vascularized wall similar to haematoma. In cut section, brownish material occupied the antral cavity. Histologically, the antral fluid occupied by a large amount of hemorrhage. The granulosa and theca interna cells appeared hypertrophied and vaculated (Mahmoud *et al.*, 2011). Hemorrhagic cysts have a thicker wall than follicular cysts and have a peculiar echo-texture resembling a hematoma (Tibary and Anouassi, 2000).

2.3.1.3. Para-ovarian cysts

Para-ovarian cysts are fluid filled structures located in the broad ligament near the ovary or uterine tube. Para-ovarian cysts could be remnants of the mesonephric (Wolfian) or paramesonephric (Mullerian) duct system. Cysts can be single or multiple, unilateral or bilateral, round or oval, measuring 0.5 to 5 cm in diameter. Their effect on fertility is not known (Tibary and Anouassi, 2000).

Grossly, these cysts are transparent, thin wall and contained clear watery fluid. Microscopically, these cysts consisted of muscular wall contained congested blood vessels and lined by simple cuboidal epithelium. Moreover, pressure atrophy of adjacent ovarian tissues also observed (Shawky *et al.*, 2004).

2.3.1.4. Ovario-bursitis /Ovario-bursal adhesion/ hydrobursitis

Ovario-bursitis is a peculiar affection of the ovarian bursa characterized by the accumulation of variable amounts of fluid and encapsulation of the ovary. The lesions are mostly unilateral and characterized by accumulation of large quantity of blood-stained or clear fluid in the bursa along with encapsulation of the ovary (Adel *et al.*, 2012). The condition is suspected when difficulty is encountered during retraction of the uterus and ovarian

palpation. In some cases, the oviduct presents a severe torsion easily identified by palpation (Tibary and Anouassi 2000).

2.3.1.5. Ovarian tumors

Ovarian neoplasms do not constitute a major cause of reproductive problems in *Camelidae*. Most of the reported ovarian tumors are teratomas. They are benign and do not seem to have any effect on follicular activity on the contra-lateral ovary. The ovarian mass presents different types of tissues cartilage, bone and hair. Dysgerminoma has been reported in the dromedary camel but is extremely rare (Tibary and Anouassi 2000).

There are three types of ovarian tumors: fibromas, dermoid cysts, and cavernous hemangiomas. The ovarian fibromas appeared as hard, whitish rounded masses, 1–2 cm in diameter, protruding from the surface of the ovary. Microscopically, the tumors showed intersecting bundles of spindle cells and connective tissue fibers. Dermoid cysts appeared sac-like structures, 1–2 cm in diameter, projecting from the surface of the ovary. Transverse sections showed hair, sweat glands, sebaceous glands, and clumps of keratinized material within the tumor which was surrounded by a thick capsule lined by multiple epidermal cell layers. Ovarian hemangiomas appeared as solid, brown masses on the surface of the ovary. Microscopically numerous thin-walled blood-filled spaces separated by fibrous connective tissue septa, giving the lesions a cavernous appearance can be seen (Adel *et al.*, 2012).

2.3.1.6. Inflammation of the Ovaries

Inflammation of the ovaries (Oophoritis or Ovaritis) may be secondary to a peritonitis or perimetritis. Ovarian inflammation and adhesions can also result from hemorrhage due to harsh manipulation of the ovaries or attempts to rupture anovulatory hemorrhagic follicles. It is characterized by loss of follicular activity because of the presence of adhesions between the ovarian surface and the surrounding tissues including the ovarian bursa, uterine tube and sometimes extending to include some intestinal loops. Ovarian inflammation and adhesions can also result from hemorrhage due to harsh manipulation of the ovaries or attempts to

rupture anovulatory hemorrhagic follicles (Tibary and Anouassi 2000). Microscopically suppurative oophoritis is characterized by liquefactive necrosis and heavy infiltration of neutrophils, lymphocytes, epithelioid cells, and histiocytes in the ovary (Adel *et al.*, 2012).

2.3.2. Diseases of Uterine tube

The most dominant pathology of the uterine tube in *Camelidae* is inflammation with occlusion or accumulation of fluid in the form of pyosalpinx or hydrosalpinx. In severe cases, the ovary and ovarian bursa may be involved and adhere to each other. However many uterine tube inflammations or occlusions are not noticed clinically. Inflammatory changes can also be seen at the level of the uterine tubal junction (uterine tube papillae) with development of micro abscesses. Prognosis for all these conditions is poor if bilateral and at best guarded if unilateral as no specific treatment. Surgical ablation in unilaterally affected animals may be considered if the other side is intact (Tibary and Anouassi 2000). The types and distribution of pathological changes in the salpinx includes:

2.3.2.1. Infundibular cysts:

The apparently normal uterine tube is soft with tortuous shape and enclosed in the mesosalpinx which arises from the lateral surfaces of the mesoovarium (Al-Eknah and Ali, 2001). Infundibular cysts are thin walled, oval, elongated or kidney shaped filled with blood stained gelatinous material with variable sizes. *Aeromonas hydrophila* and *Pseudomonas aeruginosa* are the two bacterial species isolated from infundibular cyst lesions (Adel *et al.*, 2012). The histopathology of cyst wall consisted of fibrous connective tissue and smooth muscle and lined by low columnar epithelium (Al-Eknah and Ali, 2001).

2.3.2.2. Hydrosalpinx

This condition is characterized by irregular swelling and dilatation of the salpinx as a result of the accumulation of serous fluid in its lumen. Microscopic examination revealed multiple

fluid-filled sacs lined by epithelial cells in the mucosa (Adel *et al.*, 2012). Histopathologically, cystic dialation of the lumen with flattened lining of the epithelium and inflammatory eodema on the oviduct can be seen (Shawky *et al.*, 2004).

2.3.2.3. *Pyosalpinx*

Suppurative inflammation of the salpinx usually accompanied by suppurative endometritis. It is characterized by bilateral salpingeal distension due to accumulation of pus. Microscopic examination revealed edema, focal hemorrhages and capillary engorgement of the mucosa, accompanied by heavy intra-mural infiltration of neutrophils, lymphocytes, and plasma cells. Other changes included desquamation of the mucosal epithelial lining. A mixture of pus-forming organisms *Staphylococcus aureus*, *Escherichia coli*, *P. aeruginosa*, and *Streptococcus pyogenes* are bacterial organisms associated with such lesions (Adel *et al.*, 2012).

2.3.2.4. *Chronic salpingitis*

In chronic salpingitis, the salpinx enlarged, elongated, and thickened, showing luminal distension with catarrhal exudate. Microscopic examination showed irregular thickening of the mucosal folds of the salpinx due to presence of granulomatous lesions, increased connective tissue proliferation and heavy infiltration of macrophages, epithelioid cells and lymphocytes. *Streptococcus epidermis*, *Bacillus subtilis*, *Neisseria spp.*, *P. aeruginosa*, and *Candida albicans* are common bacterial infection (Adel *et al.*, 2012). Microscopically, hyperplasia of the lining epithelium and congestion of blood vessels with mononuclear inflammatory cellular infiltration of lamina propria is noticed. Moreover, the lumen of these oviducts is filled with homogenous eosinophilic structure less substance mixed with desquamated epithelium (Shawky *et al.*, 2004).

2.3.3. Diseases of uterus

The uniqueness of camel reproductive characteristics is evident in their epithelio-chorial diffused placenta, and the exclusive location of pregnancy at the 8-15 cm long left horn of its T shaped bicornate uterus. The uterus has a body of smooth endometrium. The endometrial lining (epithelium) consists of a single layer of columnar epithelium supported by broad, highly cellular connective tissue with simple tubular glands. In addition endometrium shows irregularly raised longitudinal uterine folds and ridges. And a thick layer of myometrium surrounds these endometrial layers (Srikandakumar, *et al.*, 2001).

Beneath the epithelium is a variably loose or dense region of connective tissue, the upper lamina propria, which contain a few, evenly distributed, uterine glands or gland necks. Sub-epithelial haemorrhage is frequently observed. The number of endometrial glands is very variable and the number increases in the deeper lamina propria, which has a less-dense connective tissue. The glandular epithelium is columnar in the superficial part and cuboidal in the deeper layers. Intussusceptions of epithelial cells into gland lumens are a common artifactual finding. Some changes due to ovarian activity, especially in the presence of a functional corpus luteum, have been described (Tibary and Anouassi 2001).

Uterus of *camelidae* is known to be affected by congenital or acquired abnormalities. Amongst the most common congenital abnormalities of the uterus reported in *Camelidae* are segmental aplasia, uterus unicornis and infantilism. The acquired abnormalities of the uterus are dominated by inflammatory and infectious conditions (metritis or endometritis) (Tibary and Anouassi 2000).

Uterine infections are the most common acquired reproductive problems resulting in infertility. The most significant factors contributing to uterine infections in camelids are over breeding (i.e. excessive mating during the period of receptivity), postpartum complications embryonic death or abortion and unhygienic gynaecological examination and manipulation (Tibary and Anouassi 2001). The initial contamination of the uterus is eliminated by the natural defense mechanisms present in the uterus. However resistance of

the uterus to infection and its ability to rid itself of microorganisms is diminished in the presence of degenerative changes in the endometrium (fibrosis) or repeated heavy infection with pathogenic strains allowing establishment of the infection (Ali *et al.*, 2010).

Uterine culture yields a wide variety of non specific micro-organisms including; *Corynebacterium pyogenes*, *E. coli*, *Streptococci*, *Staphylococcus sp.*, *Klebsiella pneumoniae*, *Aspergillus spp.*, *Mucor sp.* All these organisms are ubiquitous making uterine culture results misleading if not interpreted correctly and correlated to clinical, cytological and histopathological findings (Tibary and Anouassi 2000).

2.3.3.1. Mucometra

This is characterized by accumulation of large amount of mucinous fluid in the uterine horns, distension of the uterus and thinning of its walls adhesions, and cervical occlusion. Microscopic examination showed atrophy of the endometrium, myometrium, and uterine glands and some uterine glands are cystic (Adel *et al.*, 2012).

2.3.3.2. Acute catarrhal endometritis

Grossly, the uterus is enlarged and their mucosa is severely congested and edematous. Multiple areas of petechial hemorrhages are noticed in addition to presence of thick mucoid, turbid exudate on the endometrium. The histopathological examination revealed congestion of endometrial blood vessels and lymphocytic cellular infiltration with edema in the mucosa and submucosa. Alternative areas of desquamation and hyperplasia of lining epithelium of endometrium with degeneration of endometrial glands can also be seen. Moreover, multiple areas of hemorrhages mixed with brown granules of hemosiderin pigments in the mucosa also detected (Shawky *et al.*, 2004).



2.3.3.3. *Acute suppurative endometritis*

Acute endometritis is characterised by an infiltration predominantly with polymorphonuclear leucocytes (PMNs) in the sub-epithelial zone of the stratum compactum. This endometritis can become suppurative and cause a desquamation of the endometrium. The exudate is composed mainly of PMNs with a small number of lymphocytes and plasma cells. Sub-acute endometritis is characterised by hypertrophy and thickening of the blood vessels, the subepithelial infiltration of fibroblasts, histiocytes and lymphocytes, and by a slight leucocytic infiltration around the endometrial glands (Tibary and Anouassi 2000).

The endometrium is severely congested and the uterus distended with purulent exudate. Endometrial edema, congestion, extensive epithelial desquamation, and heavy subepithelial infiltration with neutrophils and mononuclear cells can be seen microscopically. Degenerative and necrotic changes also noted in uterine glands. Various bacterial organisms including *S. aureus*, *P. aeruginosa*, *E. coli*, *Campylobacter spp.*, and *C. albicans* are associated with this inflammation (Adel *et al.*, 2012).

2.3.3.4. *Chronic endometritis*

Chronic endometritis is characterised by a predominantly lymphocytic infiltration, sometimes with the presence of plasma cells, macrophages, eosinophils or mast cells. Siderophages may be present in the postpartum uterus or following abortion or embryo loss. Chronic inflammation may be localized or generalised and may involve the stratum spongiosum. Chronic degenerative endometritis is characterised by irreversible changes including atrophy of the endometrial glands and fibrosis, reduced endometrial gland diameter and secretory activity, and pyknotic nuclei in the glandular epithelia cells. These degenerative changes are due mainly to the presence of periglandular or perivascular fibrosis. In severe cases, endometrial gland nests, with associated cystic dilation, or lymphatic cysts are observed. The severity of fibrosis is evaluated by the number of fibrotic layers present: slight (1 to 3 layers), medium (Tibary and Anouassi 2000).

2.3.3.5. Endometrial adenomyosis

The mucosa lining the uterus, the endometrium, is a highly glandular tissue that varies in thickness and vascularity with hormonal changes in the ovary and with pregnancy (Frandsen, 2005). Endometrial adenomyosis is microscopically characterized by stromal hyperplasia and proliferation of uterine glands between the myometrial muscle fibers. Some of these glands are distended with an amorphous substance while others are empty, and a few glands are cystically dilated (Adel *et al.*, 2012).

2.3.3.6. Endometrial adenocarcinoma:

Uterine neoplasias are rare in *Camelidae* Grossly, the neoplasm found in uterine horn is greatly thickened and showed firm whitish rounded masses which leading to narrowing of the uterine lumen. Cut section of this part revealed variable sized cysts contained yellowish fluid (Tibary and Anouassi, 2000).

Histopathologically, endometrial adenocarcinoma is of cystic type and evidenced by presence of many cystic like structures. These cysts are of variable sizes and round, oval or irregular in shape lined by two or multiple layers of neoplastic cells which has abundant cytoplasm and large vesicular or hyperchromatic nuclei with few mitotic figures. Multiple solid masses resulting from infiltration of neoplastic cells in abundant connective tissues stroma also observed. Moreover, accumulation of homogenous eosinophilic secretion in lumens of some dilated cysts also noticed (Shawky *et al.*, 2004).

Endometrial adenocarcinoma is malignant tumor with several, grayish, scirrhous neoplastic nodules, and coalesced masses on the peritoneal surface and wall of the uterus. Microscopic examination revealed loss of normal glandular appearance of the uterus and heavy infiltration of the uterine wall with masses of poorly differentiated neoplastic epithelial cells showing large vesicular nuclei, prominent nucleoli, and moderate mitotic activity (Adel *et al.*, 2012).

2.3.4. Diseases of cervix

Examination of the cervix is very important for the evaluation of the infertile animal as well as for the evaluation of physiological states such as prepartum dilation and postpartum involution. Congenital anomalies of the cervix due to abnormal development of the mesonephric and paramesonephric ducts (double cervix), lead to the formation of cervical cyst or segmental aplasia. The most common acquired pathological conditions of the cervix are due to local inflammation (cervicitis) or injuries during parturition or gynaecological manipulations. Other acquired anomalies of the cervix include cervical adhesions or lacerations resulting from a complication of birth or excessive trauma during manipulation (Tibary and Anouassi, 2000).

In acute cervicitis, macroscopic examination revealed mucosal congestion of the cervix with accumulation of caseous and sometimes mineralized material in its lumen. Microscopic examination showed desquamation of the lining epithelium, and cellular infiltration and focal hemorrhages in the subepithelial layer (Adel *et al.*, 2012).

Acute catarrhal cervicitis is characterized macroscopically by enlarged and edematous mucosa, congested and covered with yellowish or whitish viscous exudate. Histopathologically, congestion of blood vessels and desquamation of the lining epithelium with hyperplasia of cervical glands were detected. Focal areas of edema and hemorrhages with inflammatory cellular infiltration of cervical mucosa and submucosa mostly lymphocytes are noticed (Shawky *et al.*, 2004).

2.3.5. Diseases of Vagina and vulva

Several abnormalities of the vagina and vestibulum have been reported in *Camelidae* including segmental aplasia, persistent hymen, vaginal constriction, and presence of vaginal septum. These anomalies should be suspected when there is difficulty in penile intromission. Persistent hymen can be resolved by manual dilation of the vestibulum or by surgical

incision. Inflammation of the vagina (vaginitis) is relatively common and associated with bad management of breeding or inadequate manipulation of the vaginal cavity. These inflammations are usually noticed in the form of traumatic vaginitis following a difficult birth. The vaginal mucosa appears bruised and hyperaemic, sometimes there is a mucous secretion. Coitus can also induce traumatic injuries especially if the female is still young. Traumatic injuries of the vagina can lead to the formation of complete adhesions between the vaginal wall and development of pyometra. Vaginal prolapse occurs in *Camelidae* during pregnancy (Skidmore, 2000).

2.4. Common Bacterial Infectious Diseases in Camels

Infections in the pregnant female could lead to abortion, prenatal-neonatal loss and stillbirth. The persistence of such infections post partum is likely to contribute to the early demise of the corpus luteum (CL) in subsequent pregnancy with decrease in secretion of progesterone (P4) in the affected animal which could also lead to pregnancy failures. The bacteria colonizing the reproductive tract of the female camel (*Camelus dromedarius*) have been shown to be the major causes of reproductive disorders in this species (Mshelia *et al.*, 2014).

Brucellosis is essentially a disease of the sexually mature animals, the predilection site being the reproductive tract, especially the gravid uterus (Gul and Khan, 2007). The incidence of brucellosis in camels in different countries is seems related to breeding and husbandry practices. In Africa *Brucella melitensis* was found to be the causative agent for camel brucellosis while in the USSR the infection was due to *Brucella abortus*. The organisms were isolated in the different countries with variable incidence of success. It is claimed that young camels are resistant up to the age of 11 months and that they contract the disease from the dams on subsequent calving. If this is the case, separation of young camels at 7 to 8 months of age from positive dams might help in the control program (Merkt, *et al.*, 1990). The disease is associated with infertility and prolonged calving intervals and has considerable impact on camel production (Ismail *et al.*, 2012). Death may occur as a result of acute metritis, followed by retained fetal membranes (Gul and Khan, 2007).

Pasteurellosis and salmonellosis are also considered as causes of abortion in camels. The major infectious organisms in endometritis and metritis are *E. coli*, *Streptococcus equi* ssp. zooepidemicus, *Actinomyces pyogenes*, *Staphylococcus aureus*, *Klebsiella* spp, *Corynebacterium* species, *Proteus*, *Streptococcus* species and *Salmonella* species (Bekele, 2010).

2.5. Diagnosis of Camel Reproductive Organ Diseases

Establishing the cause of infertility in any species relies on knowing the full breeding history of the diseased animal and performing an extensive examination of the reproductive tract. The latter should include a general physical examination, palpation and ultrasonography of the reproductive tract and vaginoscopy. Transrectal ultrasonography combined with uterine flushing offer a better method to visualize echogenic intraluminal bodies, adhesions or abscesses, and to evaluate the uterine wall (Skidmore, 2000). Examination of the vaginal cavity and cervix using vaginoscopy is an important step in the diagnosis of uterine infection. Cytological examination of uterine content is a rapid and cheap way to verify the nature of any fluid present and to determine the presence of inflammatory cells. Uterine swabs should also be collected for cytology and bacteriological culture, as well as an endometrial biopsy sample to evaluate pathological changes in the endometrium (Tibary and Anouassi 2001).

Estrogen determination is indicated when follicular development is not observed after a series of ultrasonographic examinations. Multiple or single uterine cysts can be diagnosed easily by ultrasonography and fibroscopic examination of the uterine cavity (Skidmore, 2000).

2.6. Treatment for Camel Reproductive Organ Diseases

The methods of treatment of COD have varied from manual rupture to the administration of LH, HCG or products high in LH activity. The standard therapeutic approach to follicular

cyst and hemorrhagic cyst is to first induce luteinization with hCG (human Chorionic Gonadotropin) (5000 to 10,000 IU) followed 8 days later by an injection of PGF2 α or its analogue. Injection of PGF2 α or its analogue may need to be repeated 24 hours later. Females that have the tendency to develop unovulatory follicles because of failure to ovulate should be given hCG after breeding (Skidmore, 2000).

The initial contamination of uterus can be eliminated by the natural uterine defence mechanisms. However, in a proportion of females partial or complete failure of these mechanisms allows the establishment of an infection. Local immunity, phagocytosis and mechanical clearance by myometrial contractions are the major mechanisms used to clear uterine infection, and are more effective during the follicular phase of the oestrous cycle, when estrogens are high and the uterus has maximal contraction (Tibary and Anouassi, 2001)

Local treatment of uterine infection consists of uterine lavage or infusion with antibiotic solution. Uterine infusion with antiseptic solutions is progressively abandoned in favor of infusion with an antibiotic solution. The efficacy of this treatment depends on the nature of the antibiotic used, the volume infused and frequency of infusion (Skidmore, 2000). Uterine lavage is generally performed using warm, isotonic saline solution or a weak antiseptic solution. The objective of this treatment is to remove organisms and cellular debris and improve uterine clearance by promoting endometrial contraction and increased local blood flow. The ideal antiseptic solution for uterine flushing should not contain more than 1 to 3% of a 0.5% povidoneiodine solution. A lavage of the uterus with saline at the end of the treatment to remove all antiseptic from the uterus is highly recommended. Oxytocin (20 i.u.) may be given to improve uterine clearance. Uterine lavage should be carried out carefully in the case of septic endometritis to avoid complications (Tibary and Anouassi, 2001).

Uterine infection in female camels can be treated with intrauterine antibiotic bolus as in cattle or systemic injection with broad-spectrum antimicrobial agents (Bekele, 2010). However, the inappropriate use of antimicrobial agents for the treatment of infective

reproductive disorders in camels led to increased bacterial contaminations of the genital environment (Mshelia *et al.*, 2014).

2.7. Prevention of Camel Reproductive organ Diseases

Prevention of uterine infection requires a good breeding management in maiden and susceptible females and strict code of hygiene during breeding and parturition. Prevention of postpartum metritis can be achieved by regular monitoring of the periparturient female, hygienic conditions during parturition and early efficient obstetrical manipulation if needed. Any manipulation of the genital tract should be followed by local or systemic prophylactic antibiotic treatment. All post-parturient females should be monitored for signs of fever or septicemia for the first 5 days postpartum (Skidmore, 2000).

2.8. Socio-Economic Role of Camels

The economic role of the livestock sector can be classified into two: Intra-sectoral activities including wealth created due to economic activities that occurred within the livestock sector and Inter-sectoral activities - linkages of the livestock sector to other industries and domestic institutions (Fitaweke *et al.*, 2011). The camel is probably one of the most useful farm animals of the desert serving mankind for a long time in the harshest environment (Iqbal *et al.*, 2012). They have great economical potential and anticipated to make a significant contribution in solving problem in transportation, food shortage and milk supply (Al-Ani, 1998).

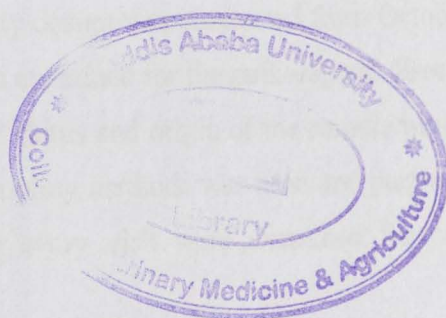
Raising camels may replace crops and other livestock in the hardest-hit arid areas of the African continent. The potential of camel in arid and semi-arid areas for generating milk or providing income from their sale was initially being considered as uneconomical. However, their value is now becoming increasingly acknowledged as a result of climate change. A typical example of this is the increasing number of camel population in pastoral community in response to changing ecology and feed availability. It seems logical to rely more on

browsing animals as the rangeland is often covered by bushes and trees and make economic use of them feasible. This has therefore, triggered changes in livestock composition. Increased aridity in Borana Zone shifted the principal stock gradually from cattle combined with small stock to camels combined with small stock (Yosef *et al.*, 2013). The camel is a more reliable milk provider than other classes of livestock in arid areas, during both dry seasons and drought years (Bekele, 2010).

Camels are important sources of cash for Somali pastoralists from sale of milk, milk products, and sales of surplus male and unproductive camels. Wealth status of Afar and Somali pastoralists are mainly determined by the number of camels owned. Afar pastoralists sell camels only when they require large amount of money (Yosef *et al.*, 2013).

2.9.Economic Importance of the Reproductive Organ Diseases

On top of the low reproductive performance of camels, reproductive disorders can lead to economic losses in terms of reduced fertility, low life time production, culling of the animal from the farm, longer calving interval and increased expenses on medication in farm animals (Al-Juboori and Baker, 2012). Several uterine disorders have been incriminated in lowered fertility in camelids. Uterine disease/disorders are the leading causes of substantial economic losses of camel production through prolonged calving intervals, reduced milk production, and culling (Mshelia *et al.*, 2012).



3. MATERIALS AND METHODS

3.1. Study Area and Study Population

The study was conducted in Akaki municipal abattoir. Akaki is 20 kms far from center of Addis Ababa. Akaki abattoir is known abattoir that supplies camel's meat for community of Addis Ababa. About 5-8 camels slaughtered every day in this abattoir.

This study was conducted on 231 female camels originated from Afar (62) and Borana(169). Afar's camels are small but extremely hardy and except for some used for salt transport, are kept almost entirely as milk producers. Borena camels (found in the Oromia National Regional State) are of intermediate size and are employed as pack, draught and dairy animals (Tezera and Kassa 2012).

Pastoral production system is observed in area of Afar and Borana in which seasonal herd mobility was observed particularly during the dry season for foraging and watering purposes, and to some extent during wet season to avoid disease occurrences (Bekele, 2010).

3.2. Study Design

Cross sectional study design was conducted from October 2013 to May 2014 and about 231 female camels were examined for the pathological disorders and associated bacterial causes. Ages, physiological status and origin of the camels were considered as variables of interest. Non probability sampling methods was used and purposely all female camels slaughtered in the abattoir during every visit were examined for presence of any reproductive organ disorder.

3.3. Sample Collection and Transportation

Abattoir visits were arranged 3 days in every week for sample collection and the other days of the week for laboratory sample processing. Per day about 5-8 camels were slaughtered in this abattoir, of which about 3-5 camels were females. Thus during the study about 3-5 camels were sampled in each day and examined for any gross pathological lesion or disorder in the reproductive organs at the abattoir.

Before slaughter antemortem examination was conducted and special focus was given for any signs of reproductive system. The opening of vulva was examined for any gross lesion and especially for sign of vaginal discharge. After slaughter, 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 lesion were sampled for histopathological examination as well as for bacteriological examination. The lesion part of the tissue is cut to the size of 2-3 cm and put in the universal bottle containing 10% buffered formalin which stabilizes the tissues to prevent decomposition. The volume of the formalin was ten (10) times larger than the size of the tissue sample. The sampled tissue then was transported to the NAHDIC for processing. This was according to (Talukder, 2007).

For bacteriological examination the surface of the tissue with the lesion was decontaminated by moderate hot application using flame of fire and then collected separately in sterile universal bottle, labeled and transported to the laboratory in icebox (Quinn *et al.*, 2004).

3.4. Sample Processing

3.4.1. Histopathological procedure

Tissue processing was conducted following the procedure described by Talukder, (2007) (Annex 1). Tissue was trimmed, fixed in 10% buffered neutral formalin, dehydrated in

ascending grades of alcohol, cleared with xylene and impregnated with molten paraffin wax. Then tissues were sectioned at 5µm thickness, spread on warm water bath and tissues were attached to albuminized glass slides. Then the slides were incubated in incubator at 60°C to avoid paraffin wax.

The sectioned tissues were then deparaffinized in three changes of xylene, rehydrated in descending grades of alcohol and stained with hematoxylin and eosin. Finally examined under microscope using 10x, 20x, and 40x magnification and photomicrographs were taken.

3.4.2. Bacteriological procedure

All bacteriological examination procedures were done according to Quinn *et al.*, (2004) (Annex 2-12). For bacteriological isolation and identification tissue was minced with sterile scissors and forceps and inoculated to brain heart infusion broth and aerobically incubated at 37°C for 24hrs. After 24hrs aerobic incubation a loopful of bacterial growth were streaked on 7% sheep blood agar and incubated aerobically at 37°C for another 24hrs. The plates then were checked for presence of growth and colonies examined for colony morphology, size, shape, and presence or absence of haemolysis. For primary identification catalase test, oxidase test, oxidation fermentation test, motility test and gram stain was conducted and suspected genera were additionally checked with selective media. Selective medium and differential medium used were includes mannitol salt agar, Edwards medium, EMB, XLD and salmonella shigella agar. After 24hrs incubation characteristic growth on selective medium were registered.

For further biochemical tests colony were inoculated in to brain heart infusion. IMViC test including indole test, methyl red, VP test and citrate test and other tests such as 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.

3.5. Data Analysis

Data were recorded, checked and coded on Microsoft Excel spreadsheet (Microsoft Corporation) and Intercooled STATA (Stata version 11) was used for descriptive analysis, Chi-square test and binary logistic regression, 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.

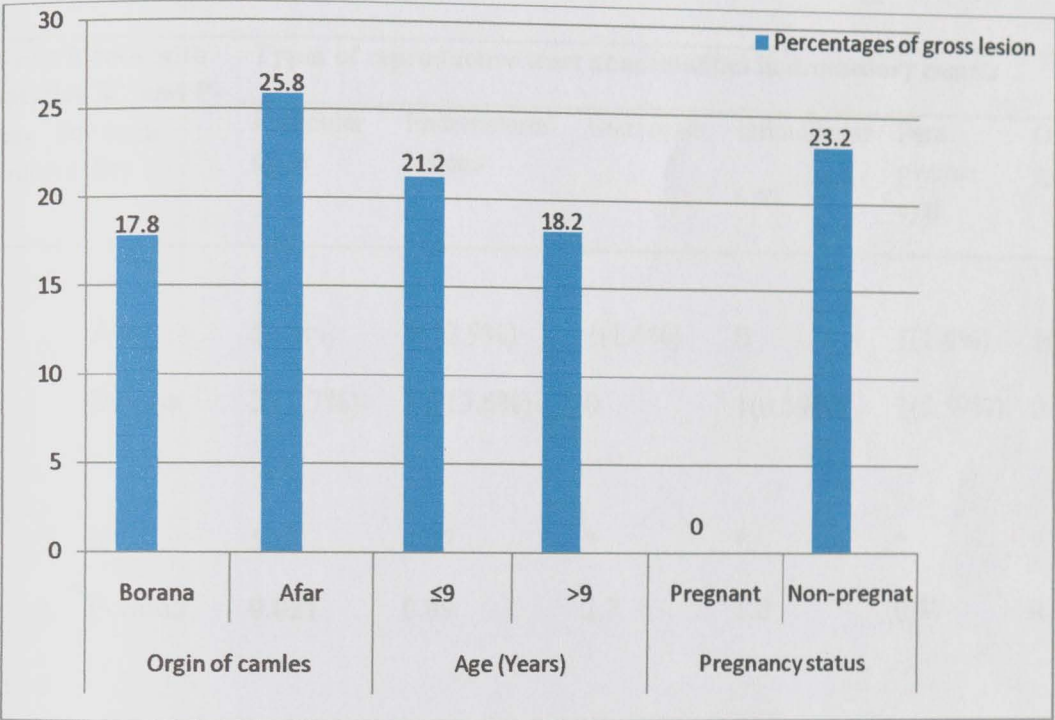
4. RESULTS

4.1. Reproductive Organ Abnormalities Encountered 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 1). Of which 30 (17.76%) 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 effect (Fig 1). This significant difference between pregnant and non pregnant camel was due to endometritis. Association of gross lesion types with age, origin and pregnancy also evaluated. The differences of the follicular cysts in origin were significant (Table 2) and level of difference was indicated (Table 3).

Table 2: Gross pathological abnormalities with their respective numbers and percentages

No.	Types of lesion	No of camels with gross lesion	Percentage (%)
1	Follicular cysts	8	3.5
2	Ovarian hypoplasia	1	0.43
3	Ovarian tumour (hemangioma)	2	0.87
4	Para-ovarian cysts	2	0.87
5	Infundibular cysts	1	0.43
6	Endometrial congestion (hemorrhages)	31	13.4
7	Uterine cysts	1	0.43
Total		46	19.9



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

Figure 4: Occurrence of reproductive disorder associated with Origin, age and pregnancy status

Table 2: Types of gross abnormalities with their respective percentages and values of significance

Factors considered with respective X ² and P-value, for each abnormality	Types of reproductive tract abnormalities in dromedary camels						
	Follicular cysts	Endometrial lesions	Uteri cysts	infundibular cyst	Para ovarian cyst	Ovarian hypoplasia	Ov
in							
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
X ²	5.37	0.02	*	*	*	*	*
P-value	0.021	0.89	2.7	1.0	0.47	0.27	1.0
(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	1.8
ancy							
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(no X² value)

Table 3: Odd ratio of risk factors with respect to different types of gross reproductive abnormality

OR, P-value, df and CI for each risk factor			Types of reproductive tract abnormalities						
with respect to each abnormality			follicular cyst	endometritis	Uterine cysts	infundibular cyst	Para ovarian cyst	Ovarian hypoplasia	Ovarian tumor
Origin	OR	Borana	1	1	1	1	1	1	1
		Afar	4.86	0.94	*	0	2.75	*	0
	CI		1.12-20.96	0.49-2.23	0	0	.17-44.7	0	0
	P-value		0.034	0.89	.99	.99	.48	.99	.99
	df		1	1	1	1	1	1	1
Age	OR	≤ 9	1	1	1	1	1	1	1
		>9	1.35	.70	0	0	1.34	0	0
	CI		0.33-5.52	.32-1.53	0	0	.08 -21.6	0	0
	P-value		.67	.37	.99	.99	.84	.99	.99
	df		1	1	1	1	1	1	1
Pregnancy status	OR	non pregnant	1	1	1	1	1	1	1
		pregnant	0	0	0	0	0	0	0
	CI		0	0	0	0	0	0	0
	P-value		.99	.99	.99	.99	.99	.99	.99
	df		1	1	1	1	1	1	1

*= very large number (this indicates no positive in the reverse category (the presence of lesion is only in one category))

4.1.1. Ovarian abnormalities and associated lesions

4.1.1.1. Ovarian hypoplasia

Ovarian hypoplasia was diagnosed in one camel. It was bilateral and the ovary was very small, oval in shape and measured 1.5 cm and 1.7 cm in length for left and right ovary, respectively. In breadth it measured 1.1 cm and 1.4 cm for left and right ovary, respectively. Grossly the surface was almost smooth; the follicles were neither seen by naked eye nor palpated externally.

Histopathologically the follicle were much reduced, the follicle were 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 stromal cells and fibroid connective tissue was also detected (Fig 2 A and B).

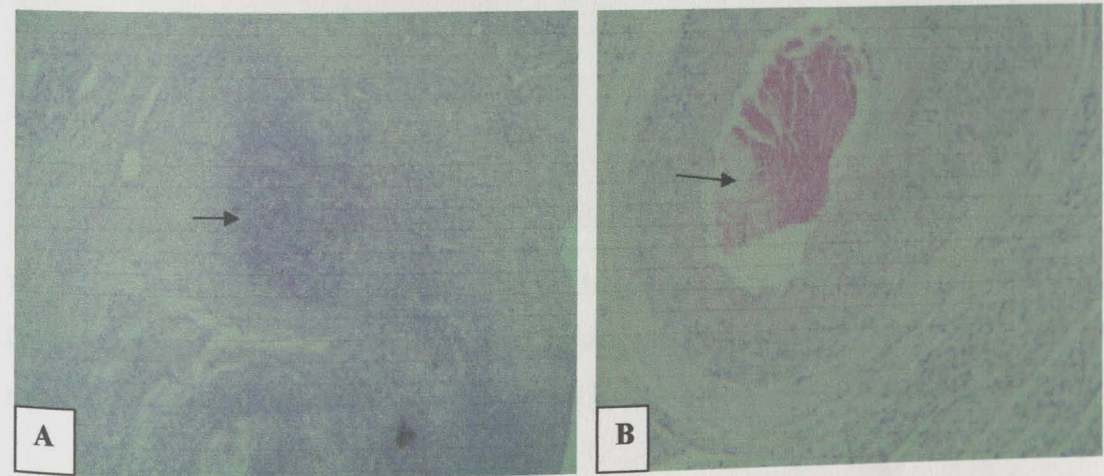


Figure 5: (A) hypoplastic ovary showing aggregation of cells (arrow) in one area without follicle formation, (B) hematoxyline eosine stain of hypoplastic ovary with congested blood vessel (arrow) seen in the center of less developed follicle (40x magnification) (H&E stain).

4.1.1.2. Ovarian follicular cysts

Ovarian follicular cysts were detected in 8(3.5%) camels. These cysts contained clear fluid, enclosed in thin layer of connective tissue and granulosa cells. These cysts were externally looks pale and vascularized. The sizes of these cysts ranged between 23mm-36mm and all were found on the left side of the uterus (Figure 3, A). 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 3, B).

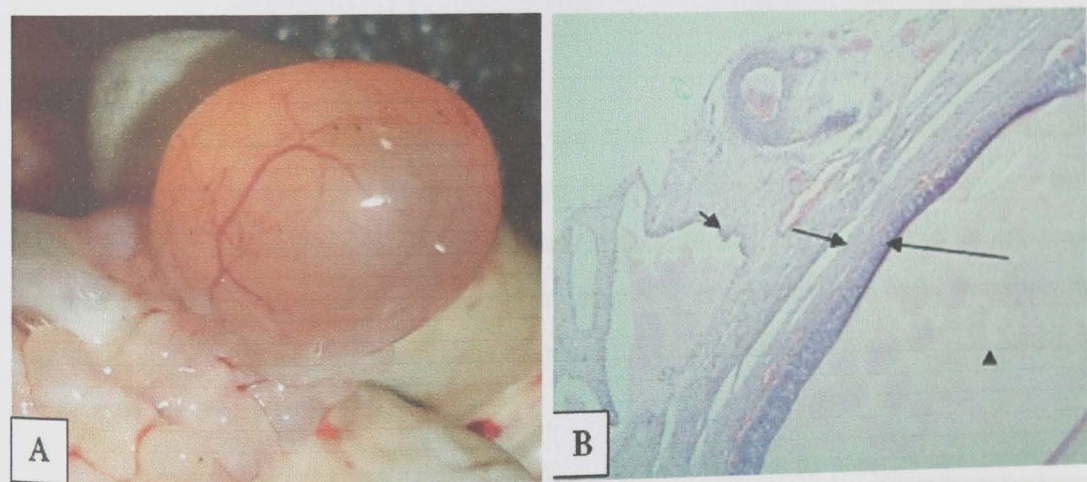


Figure 6: (A) Follicular cyst (Gross) (B) histologic features of follicular cyst (H&E stain) (arrow head = proteinaceous fluid, short arrow=theca externa & congested blood vessels, medium arrow=theca interna, long arrow= thin granulosa cells) (40x magnification) (H&E)

4.1.1.3. Ovarian tumor

Of the examined 198 ovaries (from non pregnant) two showed unilateral ovarian tumors (hemangioma) which were found on the left ovaries. Grossly they were well demarcated, firm in consistency, 1.1 mm and 0.8 mm in length and width, respectively, circular in shape and black-red in color. The outer surfaces of the neoplasms were smooth with prominent

vessels and blood oozed freely from the cut surfaces (Fig 4, A). The remaining ovarian tissue was pale to gray and various numbers of corpus lutea and follicles were found in the ovaries.

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 it was empty space or edematous fluid filled. The vascular spaces were separated by connective tissue stromal cells. There was also a focal area of cellular pigmentation and a few numbers of macrophages in the stromal cells (Fig 5, A and B, page 34).

4.1.1.4. Para-ovarian cyst

Out of 231 camels 2 (0.87%) had Para-ovarian cyst. Para-ovarian cysts are those cysts that located near the ovary attached to the broad ligament. Grossly these cysts are clear fluid filled cysts and they measured about 5-8 mm in size. In this study para-ovarian cyst were detected unilaterally only on the left sides of the ovary. In one case two cysts was appeared simultaneously on the one side of the ovary and in other case only one cyst was found on one side of the ovary (Fig 4, B).

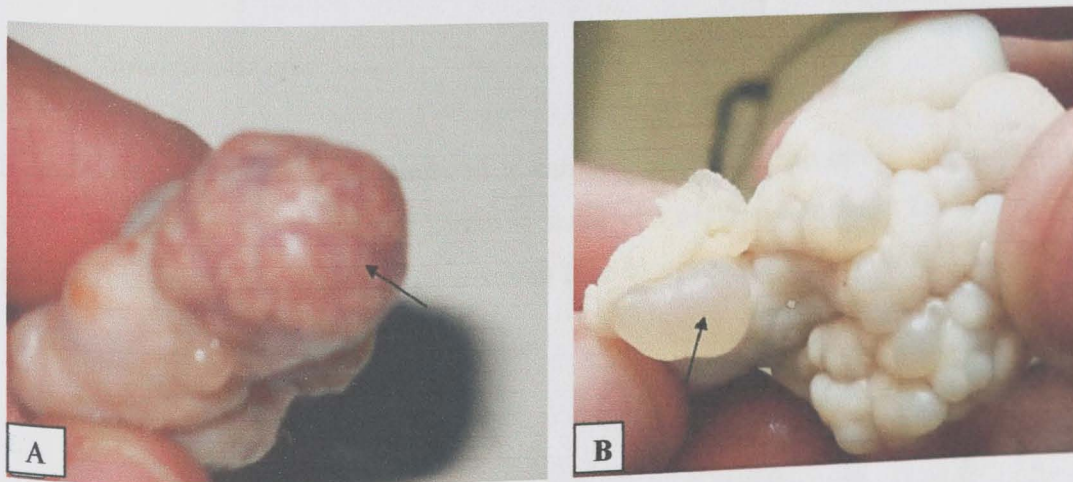


Figure 4. (A) ovarian tumour (Gross), (B) Para-ovarian cyst (gross)

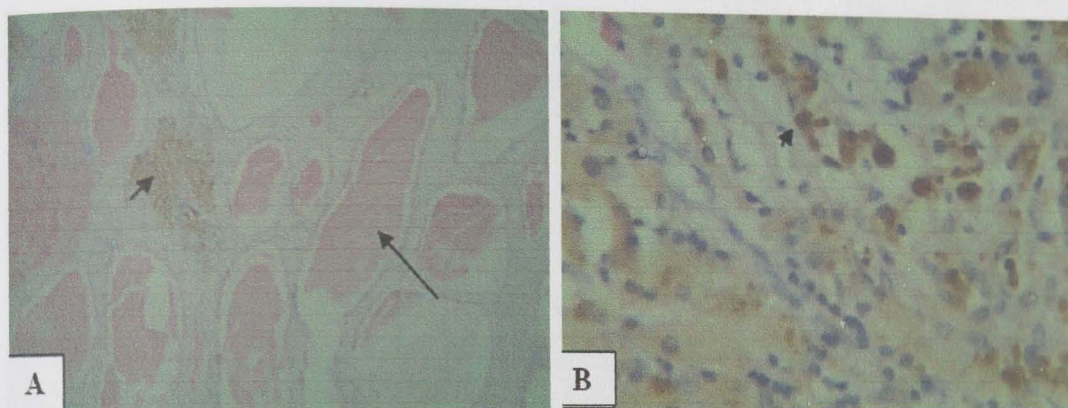


Figure 5: (A) histopathology of hemangioma, with blood filled cavity (cavernous) long arrow and pigmentation (short arrow) (B) pigmentation in hemangioma (arrow) (H&E stain) (40x magnification).

4.1.2. Uterine abnormalities and lesion characterization

4.1.2.1. Uterine cyst

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 (Fig 6, A).

4.1.2.2. Infundibular cyst

Infundibular cyst was also encountered in camel. It was a clear to serous fluid filled cyst which measured about 32mm and 24mm in length and width, respectively. The wall of this cyst was externally thin, pale and clear-serous fluid was oozed during incision (Fig 6, B).



Figure 6: (A) uterine serosal cyst(gross), (B) infundibular cyst(gross)

4.1.2.3. Endometrial lesion

In this study uterine hemorrhage and endometrial congestion were found with high incidence rate than other pathological cases accounting for 13.4% (N=31). Of which 5.2% and 6% were acute and chronic endometritis respectively. Grossly these uteruses were congested and the congestion covers some parts of the endometrium, and in other cases the congestion and pin point hemorrhages were distributed over the entire parts of the endometrium and as well the serosal parts of the uterus, including the body and horns of the uterus, even in some cases it was extended to cervix and vagina. In other cases it covers only some parts of the endometrium (Fig 7, A).

From Afar area four camels were found with acute endometritis and other four camels with chronic endometritis. Whereas from Borana 8 and 10 camels were found with acute endometritis and chronic endometritis respectively. In acute endometritis grossly visible congestion of the endometrium and enlarged (increased in width) uterine was recorded. Histopathologically hemorrhage and infiltration of the polymorphonuclear cells (neutrophils) in endometrial layer of the uterus was detected (Fig 7: B and C). In case of chronic endometritis microscopically the periglandular cuffing of lymphocytes, infiltration

of macrophages, hyperplasia of endometrial glandular cells, suppuration with infiltration of neutrophils in myometrium and formation of edema in myometrium were detected (Fig 8: A, B and C).

Endometrial calcification was found in one case of the animal. Grossly it was not easy to detect the uterine calcification case and only enlargement and tough consistency was detected. Histopathologically calcification of the endometrium and infiltration of macrophages was found (Fig 8, D).

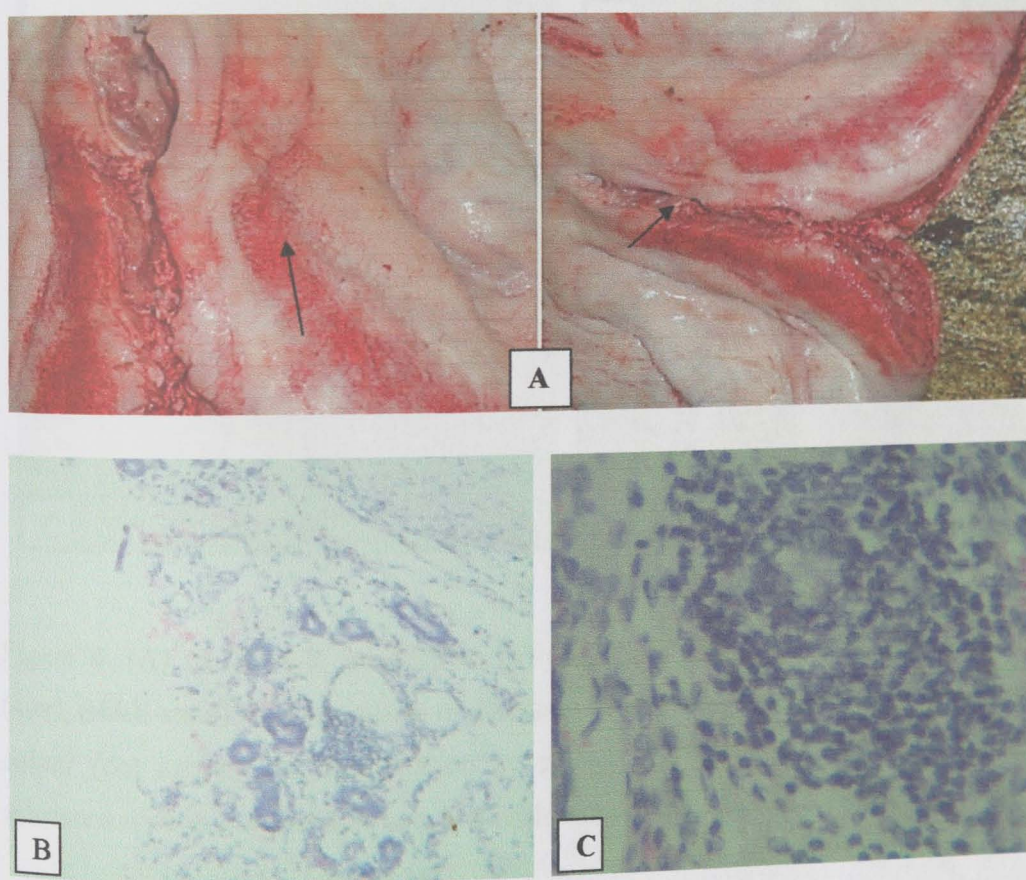


Figure 7: (A) Gross pathology of endometrium with hemorrhage and congestion, (B) hemorrhage in endometrium(40x), (C). periglandular cuffing of the lymphocytes in endometrium(40x),

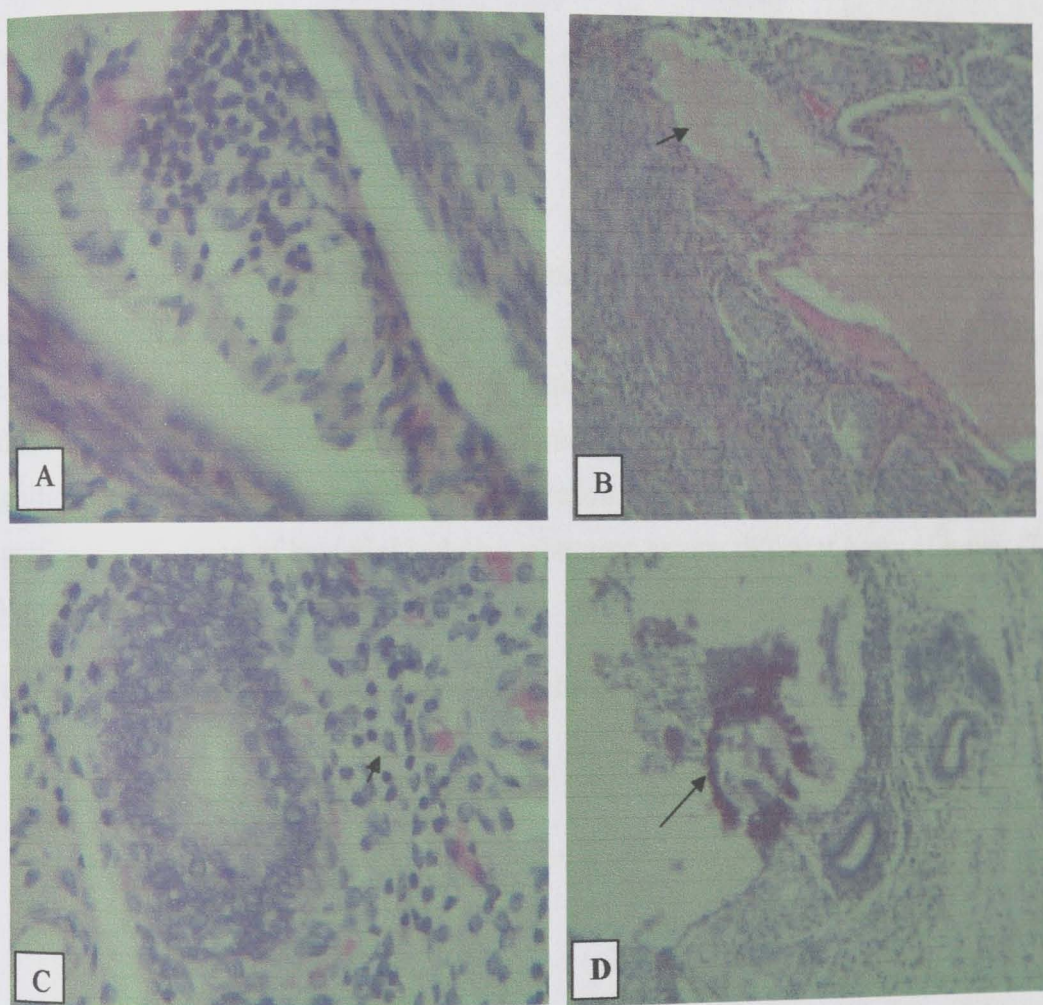


Figure 8: (A) neutrophile infiltration in myometrium(40x), (B) Edema in myometrium (40x), (H&E stain) (C) hyperplasia of glandular cells in which cells are enlarged and pale in colour (dot arrow) and infiltration by plasma cell (long arrow)(40x), (D) Calcified substance with in the endometrium, and infiltration of the macrophages(40x) (H&E stain)

2. Bacterial Isolation

of the 31 cases of endometritis examined, different types of bacteria were isolated from (83%) and the main bacterial isolates were *Escherichia coli* (38.7%) and *staphylococcus* (35.5%) species (Table 4).

Of the 23 camels with endometritis from Borana and which uterine samples were cultured for bacteriology 18 camels were positive for one or more bacterial species. Except *Salmonella* species and *proteous vulgaris* which were isolated only in acute endometritis, other bacteria were isolated from both acute and chronic endometritis. There were no significant difference found in bacterial isolation with difference in age and origin, but significant difference of bacterial isolation were seen among different types of lesion (Table

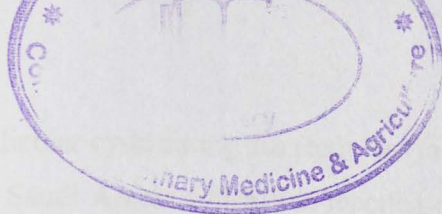
Table 4: Uterine bacterial isolates from *Camelus dromedarius* with endometritis

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

Table 5: Association of bacterial detection with origin, age and types of lesion

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

5. DISCUSSION



Reproductive organ diseases and disorders of female camels are important problem affecting the reproductive performance. Study of the pathological disorders and infection of the reproductive disease is very important in evaluation of the reproductive efficiency. The reproductive disorders of female camels was reported in different countries including Egypt, Saudi Arabia, Iran, Nigeria and Sudan (Nourani and Khodakaram, 2004; shawky, 2004; Ali *et al.*, 2009; Adel *et al.*, 2012; Mahmoud *et al.*, 2011 and Mshelia *et al.*, 2014). But no study yet carried out in Ethiopia concerning abattoir based gross, histopathological and bacteriological examination of the reproductive organ diseases. The present study thus attempted to recognize the important reproductive organ abnormalities of she camels.

In this study the statistical difference was observed in the occurrence of the reproductive organs disorder between the pregnant and non pregnant camels ($P < 0.05$). This could be due to difference in physiological status between the two categories. When related with the previous study the present study detects the low rate of occurrences of the reproductive organ disorders. The incidence of the ovarian disorders in this study was 4.8% including ovarian hypoplasia, ovarian hemangioma and ovarian follicular cyst. This finding of the ovarian abnormality was lower than the finding in the Saudi Arabia and in the Egypt which was 17.14% and 40% respectively (shawky *et al.*, 2004 and Adel *et al.*, 2012). A wider variation in the incidence of ovarian disorders in camels was reported by different authors, that the difference in ovarian abnormality could be due differences in nutritional seasonal and genetics (Adel *et al.*, 2012).

Significant difference in follicular cysts was recorded in the two regions (Borana and Afar) of the present study. This difference might be due to variation in breeding practices of camels from the two areas, as camels are induced ovulators by mating. Deen (2013) reported the more troubling fact that the behavior of female camels towards male was not correlated with ovarian follicular status. But Adel *et al.*, 2012 justified that the probable variation in the ovarian cysts is due to variation in the heat stress.

The finding of ovarian follicular cysts during this study was in agreement with the reports in the eastern region of the Saudi Arabia which was reported as 3.81% (Adel *et al.*, 2012). Incidence of the follicular cysts in the present study was 3.5%, which is slightly lower than in Kalyoubia, Egypt (5.2%) (shawky *et al.*, 2004), but it is higher than incidence reported in the Iran which was 2.08% (Nourani and Khodakaram, 2004). The maximum size of follicular cyst reported in Egypt (4.5cm) is higher than the maximum size reported in this study (3.6cm). The variation in the size of the follicular cyst could be due to variation in the time duration of the formation of the cyst.

Absence of ovarian follicular activity in hypoplastic ovary is a frequent condition in *Camelidae* (Shawky *et al.*, 2004). The present report of the bilateral ovarian hypoplasia (0.43%) is lower than the reports in eastern, western and central parts of the Saudi Arabia which was 1.95% in the three areas (Adel *et al.*, 2012). This study has similar results with the ovarian hypoplasia reported in the Egypt which was 0.4% (shawky *et al.*, 2004). The ovarian hemangioma is one of the ovarian tumors previously recorded in camels. The finding of ovarian hemangioma in this study was in concordant with the finding in the eastern region of the Saudi Arabia (Adel *et al.*, 2012). But the incidence reported in the Saudi Arabia (0.48%) was a little bit lower than the present study finding (0.87%). The finding or exploring of the causes of ovarian tumor needs further study.

Para-ovarian cyst was reported from camels in Egypt, Al-Ahs (Saudi Arabia) and Iran with prevalence of 1.8%, 0.5% and 2.08% respectively (Shawky *et al.*, 2004, Nourani and Khodakaram, 2004 and Mahamoud *et al.*, 2011). The reports in the Egypt and Iran were higher than the present study whereas a report in the Saudi Arabia is lower. The size of the cysts presented in this report is lower than the size reported in the Egypt.

The infundibular cyst previously reported in the eastern, western and central region of Saudi Arabia was 3.89% (Adel *et al.*, 2012). In other study in the Saudi Arabia incidence of infundibular cyst was reported as 1.3% (Mahamoud *et al.*, 2011). The size of the cysts reported in this study is lower than the size of infundibular cysts reported in the Saudi

Arabia (Al-Eknah and Ali, 2001). The present study had has limited in the finding of the cause of infundibular cysts. Possible of cause of the infundibular cyst needs further study.

The presence of cysts on the uterine serosa was in correspondence with the finding of Skidmore, 2000. These cysts can also be encountered on the uterine serosa or in the broad ligament and has usually no effect on fertility if they are small (Skidmore, 2000). Calcification of the endometrium is the first report in camels in this report. Calcium salts, usually in the form of phosphates or carbonates, may be deposited (calcium buildup) in dead, dying, or normal tissue (Donnald *et al.*, 2002).

Pathological lesion of endometrium was the common reproductive problem reported in previous study. The present study also determined endometritis as a major reproductive organ problem in Borana and Afar region. The incidence of acute endometrits in the present study was 5.2%, which is lower than the reports in the Saudi Arabia (9.56%) (Adel *et al.*, 2012). The microscopic finding of the chronic endometrits was similar to the reports in the Egypt (shawky *et al.*, 2004). A very large incidence of the endometritis (45.9%) was also reported in the Saudi Arabia (Ali *et al.*, 2010).

The bacteria colonizing the reproductive tract of the female camel (*Camelus dromedarius*) have been shown to be the major causes of reproductive disorders in this species (Mshelia *et al.*, 2014). A uterine infection is the most commonly acquired reproductive problem and are regarded as the most important cause of infertility in camelids. Bacterial isolation from endometritis was reported by many authors in different country. The reports of *Escherichia coli* from chronic endometritis and isolation of the *Proteous vulgaris* in acute endometritis in this study was similar with the reports in the Saudi Arabia (Adel *et al.*, 2012). Similarly the isolation of *Staphylococcus species*, *Corynebacterium species* and *Salmonella species* from endometritis also in line with the reports in the Sudan (Yagoub, 2005). There were about 45 bacteria of 7 genera isolated from the endometritis in this study, which is in correspondence with the reports of Mshelia *et al.* (2014) in Nigeria who reported about 69 bacteria of 5 genera.

Isolation of the *streptococcus* in endometritis also in line with reports of Abdel-Aziz and Nasser, 2013 in Saudi Arabia. Tibary and Anouassi 2001 also reported, isolation of *Enterobacter aerogenosa* and *Salmonella* species from uterine lesion of the camels. Evidence implicating bacterial infection as a cause of endometritis has been reported (Mshelia *et al.*, 2014). But the role of these bacteria as a cause of endometritis and as a cause of infertility in camels from Ethiopia needs further study.

6. CONCLUSION AND RECOMMENDATION

Different pathological abnormalities of reproductive organs of female camels were recorded and Gross lesions of affected organs were processed in the laboratory for histopathological characterization and bacterial isolation. The study used Afar and Borana camels slaughtered in Akaki abattoir. Endometritis and ovarian cyst were the major reproductive organ problem recorded during the study period. About seven types of bacterial genera were identified from endometritis, in which *E.coli* and *staphylococcus* species were the major bacterial isolates, identified. This study was limited to the histopathological finding and bacterial isolation. The finding of the present study suggests frequent detection of diseases of female camel's reproductive organ to address the corrective, control and prevention methods for improving of the reproductive performance of this species. Based on the above conclusion the following recommendations are forwarded.

- Further studies needs to illustrate the clinical features of these diseases.
- 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 advanced molecular techniques for localized specific organism in lesions with causal relationship should be conducted.
- There is scarcity information about camels in Ethiopia and collaborative research activities should be practiced to understand the productivity of *Camelus dromedarius* and devise proper intervention mechanisms.

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8. APPENDIX

Annex 14: Hematoxyline eosine stain procedure (Talukder, S., (2007)

Staining Procedure:

1. Deparaffinize slides in 3 changes of xylene for 3 minutes each.
2. Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
3. Place in Hematoxyline for 8 - 15 minutes.
4. Rinse in tap water until water runs clear.
5. Decolorize in 1% acid alcohol, 3 - 6 quick dips. Check differentiation microscopically: Nuclei should be distinct; Cytoplasm should be uncolored.
6. Rinse in tap water until ripples disappear from slides.
7. Dip in Bluing Agent, 3 - 5 long dips.
8. Wash in luke-warm tap water for 5 minutes (37-40°C.)
9. Stain in Eosin for 30 seconds - 2 minutes.
10. Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.
11. Clear in 3 changes of xylene for 2 minutes each.
12. Mount coverglass.

Results:

Nuclei	Blue
Cytoplasm and other constituents	Varying shades of red
Blood	Bright red
Cartilage and calcium deposits	Dark blue

Annex 15: The Gram Stain (Carter and Wise (2004))

Procedure:

From a liquid culture, take a loop full of bacteria emulsify it in a small drop of water or saline on the slide. This should be a thin, not milky, suspension or it will not stain properly. Air dries the slide. This is done automatically in the virtual module.

To begin:

1. Heat fixes the slide: click on the Bunsen burner; pass the slide gently two or three times (1-2 seconds) through the flame. Do not overheat - this will cause distortion of the cells.
2. Flood the slide with crystal violet for 1 minute
3. Rinse with H_2O
4. Flood the slide with iodine for 1 minute
5. Rinse with H_2O
6. Decolorize with alcohol for 5-10 seconds
7. Rinse with H_2O
8. Flood the slide with safranin for 1 minute
9. Rinse with H_2O
10. View slide under the microscope

Annex 16: Catalase test (Carter and Wise 2004)

This test detects the enzyme catalase that converts hydrogen peroxide to water and gaseous oxygen.

Procedure:

A. Slide method

- With an inoculating needle pick a pure colony and place on a clean glass slide.
- Add a drop of 3% H_2O_2 over organisms on slide
- Test cannot be applied if blood agar is used

- Do not reverse the order of procedure as false positive result may occur.
- Do not mix with inoculating needle or loop. Mixing of culture and H_2O_2 is not necessary

B. Tube methods

- Directly add 1.0 of H_2O_2 to an 18 to 24 hrs heavily inoculated pure agar slant culture.
- Observe for immediate bubbling and record the result.

Interpretation

Positive result: immediate bubbling, easily observed

Negative: no bubbling

Annex 17: Oxidase test (Carter and Wise, 2004)

The oxidase test is based on the bacterial production of an oxidase enzyme. The oxidase reaction is due to the presence of a cytochrome oxidase system, which activates the oxidation of reduced cytochrome by molecular oxygen. This in turn as an electron acceptor in terminal stage of electron transfers system. The cytochrome is an iron containing hemo proteins that acts as the last link in the chain of anaerobic respiration by trasfering electron (hydrogen) to oxygen with the formation of the water or H_2O_2 .

Oxidase reagents

Tetramethyl-p-phenylenediamine dihydrochloride (1%water solution) – **kavac's** reagent

Dimethyl-p-phenylenediamine dihydrochloride (1%water solution) - **Gordon and Mcleod's** reagent.

Test procedure:

a. Direct plate procedure

- Add 2-3 drops of oxidase reagent directly to the a few suspected colonies growing on plate medium. Do not flood the entire plate.
- Do not invert the plate
- Observe color change after 15-30 seconds (Kovac's reagent)

b. Indirect paper procedure

- Place a 6cm² pieces of Whatman no. 1 filter paper in petri dish
- Add 2-3 drops of Kovac's reagent to center of the paper
- Smear loop full of a suspected colony on to the reagent impregnated paper in a line 3-6 cm long
- A positive color reaction occurs within 5-10 seconds

Interpretation

Oxidase positive: the colonies form dark blue color after few seconds

Oxidase negative: no color formation

Annex 18: Oxidation fermentation test (OF test)

This test is used to determine oxidative fermentative metabolism of carbohydrate by bacterium. The medium is usually semisolid and contains glucose as a test agar and bromothymol blue as a pH indicator.

Principle

The utilization of carbohydrate by bacterium occurs metabolically by one of the two processes, fermentative or oxidative. Some bacteria are capable of metabolizing a carbohydrate under the aerobic condition while others produce acid both aerobically and anaerobically, and are termed as facultative anaerobes. Fermentation is an anaerobic process. By fermentation processes carbohydrate is split into two triose carbon molecules

which are further converted to 1, 2, 3 or 4 carbon compounds: the key intermediate product in pyruvic acid. Fermentation produces a higher acidity than oxidative metabolic processes.

Procedure

Two tubes of OF medium are heated in a beaker of boiling water immediately before use in order to drive off any dissolved oxygen. Each tube is inoculated with unknown organisms using straight needle. One tube of each pair is covered with 1cm layer of sterile mineral oil or melted paraffin, leaving the other tube open to the air. Incubate at 35°C and examine for several days.

Interpretation

	<u>Open tube</u>	<u>sealed tube</u>
Unreactive	green	green
Oxidative	yellow	green
Fermentative	yellow	yellow

Acid production is indicated in the medium by the appearance of the yellow colour.

Annex 19: Urease test

It is to determine the ability of the organisms to split urea, forming two molecules of ammonia by the action of the enzyme urease.

Procedure:

Urea agar/broth inoculated with a loop full of pure culture of the test organisms and incubates at the 35°C for 18-24 hrs.

Interpretation

Organisms that hydrolyse urea rapidly may produce positive reaction within 1-2 hrs less active spp may require 3 or more days.

Rapid urea splitters – red (pink) color throughout the medium

Slow urea splitters – Red (pink) initially in slant only gradually converting the entire tube.

No urea hydrolysis – medium used original yellow color.

Annex 20: Indole test:

The test is used to determine the ability of the organisms to split indole from tryptophan molecules.

Principles:

The indole test is based on the formation of red colour complex when indole reacts with the aldehyde group of p-dimethyleaminobenzaldehyde. This is the active chemicals in the Kovac's reagents.

Procedure

Inoculate the indole media with the test organisms and incubate at 37°C for 18- 24hrs.

Add 5 drops of Kovac's reagent directly to incubated tube and shake the tube gently. Keep it for a minute and see colour change at surface of the media.

Interpretation

Positive – pink to red colour at the interface of the reagent and the broth

Negative- no colour change (remains bright yellow)

Annex 21: Triple Sugar Iron Agar

Principle

The configuration of the slant agar result is essentially two reaction chambers within the same tube:

The slant portion exposed throughout its surface to atmospheric oxygen is aerobic.

The lower portion called butt or deep is protected from air and is relatively anaerobic

Procedure

Once isolated colony from selective medium is touched with wire loop. A tube is inoculated in middle of the agar. On the withdrawal of the straight wire the entire slant is streaked. The medium is incubated at 37°C for 18-24 hrs.

Interpretation

Alkaline slant/ alkaline deep (K/K) - no carbohydrate fermentation

Alkaline slant/ acid deep (K/A)- glucose fermented either lactose or sucrose is not fermented

Alkaline slant/ acid (black) deep (K/A/H₂S) - glucose fermented either lactose or sucrose is not fermented, H₂S produced.

Acid slant /acid deep (A/A)- glucose, lactose or sucrose fermented

Annex 22: methyl red test

Methyl red is a quantitative test for acid production (mainly used in the identification of enterobacteriaceae) requiring positive organisms to produce strong acid (lactic, acetic and formic) from glucose through the mixed acid formation pathway. Since many species of enterobacteriaceae may produce sufficient quantities of strong acid that can be detected methyl red indicator during the initial phase of incubation, only organisms that maintain this low pH after prolonged incubation (48-72 hrs) overcoming the pH buffer system of the medium can be called methyl red positive.

Interpretation

The development of stable red color on the surface of the medium indicates sufficient acid production to lower the pH to 4.4 and constitutes a positive test. Since other organisms may produce lesser quantities of acid from the test substrate intermediate orange color between yellow and red color may develop this does not indicate a positive test.

Annex 23: Voges Proskauer(VP)

To detect acetyl methyl carbinol formed by bacterial metabolism, a product of the butylenes glycol.

Principle

Pyruvic acid the pivotal compound formed in the fermentative degradation of glucose is further metabolized through a number of metabolic pathways depending on the enzyme system possessed by different bacteria. One such pathway results in the production of the acetoin (acetyl methyl carbinol) a neutral reacting end product. Some organisms produce acetoin as the chief end product of glucose metabolism and form fewer quantities of mixed acid. In the presence of atmospheric oxygen and 40% potassium hydroxide acetoin is converted to diacetyl and alpha naphthol serves as a catalyst to bring out a red color.

Procedure

Incubate a tube of MR/VP broth with a pure culture of the test organisms. Incubate for 24hrs at 35⁰c. at the end of this time add 0.6ml of 5% alpha naphthol, followed by 0.2ml of 40% KOH. It is essential that the reagent added in this order. Shake the tube gently to expose the medium to the atmospheric oxygen and allow the tube to remain undisturbed for 10-15 minutes.

Interpretation

A positive test is represented by the developments of the red color 15 minute or more after addition of the reagents, indicating the presence of the diacetyl, the oxidation product of acetoin. The test should not be read after standing for over one hour. Because negative VP culture may produce a copper like colour potentially resulting in a false positive reaction.

Annex 24: Citrate utilization

To determine the utilization of the citrate as a sole source of the carbon. Sodium citrate is a salt of citric acid, a simple organic compound found as one of the metabolites in the tricarboxylic acid cycle (Krebs cycle). Some bacteria can obtain energy in the manner other than the fermentation of the carbohydrates by utilizing citrate as the sole of the carbon sources. The measurement of this characteristic is important in the identification of the many members of the enterobacteriaceae.

Principle

The utilization of the citrate by a test bacterium produce an alkaline by products. The medium includes sodium citrate an anion as the sole carbon and ammonium phosphate as the source of the nitrogen. Bacteria that can utilize citrate can also extract nitrogen from the ammonium salt with the production of the ammonia NH_3 leading to alkalization of the medium from conversion of the NH_3^{2+} to ammonium hydroxide (NH_4OH), pH 7.6 is the indicator.

Test procedure

All well isolated colony is picked from the surface of the primary isolation medium and inoculated as a single streak on the slant surface of the citrate agar tube. The tube is incubated at 35°C for 24-48hrs.

Interpretation

A positive test is represented by the development of a deep blue color within 24-48 hrs indicating the test organisms has been able to utilize the citrate containing in the medium with the production of the alkaline products. A positive test may also be read without a blue color if there is a visible colonial growth along the inoculation streak line. This is possible only if carbon and nitrogen have assimilated. A positive interpretation from reading the streak line can be confirmed by incubating the tube for additional 24 hrs when a blue color usually develops. Negative and uninoculated control citrate media is green in color.

Annex 25: Coagulase test

This correlates well with pathogenesis. Fresh or reconstituted commercial freeze-dried rabbit plasma is the reagent used. The rabbit plasma contains fibrogen that is converted to fibrin by staphylococcal coagulase enzymes.

Tube coagulase test: 0.5 ml of rabbit plasma is placed in small 7mm test tube. Two drops of an overnight broth culture added. The tube is rotated gently to mix the contents and then incubated at 37⁰c preferably in water bath. A positive test with clotting of plasma can occur in 2-4 hours.

Annex 26: Calculating the age of a camel from its teeth (Ernest, 2008)

It is useful to know the age of camels for four reasons:

- It helps the camel keeper decide whether to breed the camel.
- Age is important when buying or selling camels.
- Age is important when selling camels for slaughter – the older the camel, the tougher the meat.
- Age is important in monitoring how well a camel is growing.

Aging camels using permanent front teeth – incisors ('jembe')

- 1st pair erupts at 4.5 to 5 years.
- 2nd pair erupts at around 6 years.
- 3rd pair erupts at 7-8 years.
- The amount of wear of the teeth determines the age thereafter.

NOTE - Severe wear normally seen in camels at age 4.5 - 5 years can be confused with that seen in very old camels. In the younger mouth the worn down deciduous teeth will be seen as smaller stumps and most, if not all, will be loose. In the older camel the stumps will be larger and firm and the older camel's gums may be yellowish rather than pink. A golden

rule is to look at the camel before looking in the mouth - if the camel looks young it probably is young.

Teeth at Nine Months

Eruption of central deciduous incisors occurs at birth to 14 days, laterals at 4 - 5 weeks and corners at 6 - 12 weeks, and, commencing with the centrals, some wear may be occurring by 6 months. Deciduous canines and upper premolars 1, 2 & 3 and lower premolars 1 & 2 are all obvious by 6 months. Upper deciduous corner incisors may have shed from females by 12 months.

Teeth at One and One Half Years

A full set of deciduous teeth are present by one year of age and all lower incisors are in wear. All deciduous teeth are fully functional at eighteen months. Upper and lower molar 1's (permanent teeth) erupt between 12 and 15 months and are proud of the gums by 18 months.

Teeth at Three and One Half Years

At 2 - 2.5 years deciduous incisor teeth show progressive wear and separation. Also, all molar 1's come into wear at 2 - 2.5 years and all molar 2's are about to erupt.
At 3 - 3.5 years deciduous incisors are well worn and separated and some may be loosening and all molar 2's have erupted.

Teeth at Fifteen Years

At 4 years deciduous incisors have worn down to small irregularly shaped, loose stumps.
At 4.5 - 5 years lower deciduous premolars are shed and are usually not replaced. Permanent central incisors erupt behind the deciduous stumps (if latter still present).

At 5 - 5.5 years upper permanent premolars 2 & 3's and permanent lower premolar 2's

erupt. Deciduous lateral incisors are shed. Upper and lower molar 1's and 2's are in wear and molar 3's about to erupt.

At 6 - 7 years upper corner permanent incisors (not found in every camel), upper and lower permanent canines and upper permanent premolars have all become apparent. Permanent central and lateral incisors are in wear, and lower permanent corner incisors erupt and develop. All upper and lower permanent premolars and molars are in wear by 7 years.

At 8 years all permanent teeth are present and in wear. Premolar 1's, when present, are darkly stained due to plaque and scale. Canines, particularly in males, are large and powerful.

8 - 15 years sees progressive wear of the teeth at a rate related to the food consumed and its content of abrasives. Definite separation of the permanent incisors usually commences at about 15 years.