

## Pathological and Bacteriological Study on Abnormalities of Female Internal Reproductive Organ of *Camelus dromedarius* Slaughtered at Akaki Abattoir, Ethiopia

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**Abstract:** A cross-sectional study design was devised to characterize the pathological lesions and to isolate bacterial associated with those pathologies in *Camelus dromedarius* slaughtered at Akaki abattoir. Reproductive organs of 231 female dromedary camels consisting of 169 from Borana and 62 from Afar were grossly inspected and tissue and swab samples were collected for histopathological and bacteriological studies. Histopathology and bacterial culture were done following standard protocols. A total of 46 (19.9%) camels were found with reproductive organ disorders consisting of follicular cyst (3.5%), ovarian hypoplasia (0.43%), ovarian tumor (0.87%), para-ovarian cyst (0.87%), infundibular cyst (0.43%), uterine serosal cyst (0.43%), endometritis (13.4%) and endometrial calcification (0.43%). Uterine abnormalities were the major disorders recorded in this study accounting for 13.4%. There were no statistically significant differences in occurrences of abnormalities at different age groups and areas of origin. However, there was significant difference between pregnant and non-pregnant camels with higher occurrence in the latter groups. From camels with endometritis 83% were positive for different types of bacterial isolates. *Escherichia coli* (38.7%) *Staphylococcus* spp. (35.5%), *Streptococcus* spp. (25%), *Enterobacter aerogenosa* (16.1%), *Corynebacterium* spp. (12.7%), *Proteous* spp. (9.7%) and *Salmonella* spp. (6.5%) were bacterial isolates identified in this study. Considering the importance of *Camelus dromedarius* among the pastoral society of the country, there should be collaborative studies pertinent to reproductive health to improve production and productivity in this species.

**Key words:** Dromedary camels • Endometritis • Reproductive organs pathology

### INTRODUCTION

The reproductive efficiency of dromedary camels is very low [1] and is a major problem in camelids [2]. Uterine abnormalities take the larger share of reproductive tract abnormalities in camels [2]. The short breeding season is an important factor for the low reproductive performance of dromedary camels which is due to availability of food. It remains as a major obstacle to the growth of population of dromedaries [3]. Anatomical abnormalities and pathological processes of female reproductive tract have

also been reported as the main causes of infertility [4]. Infectious diseases of the reproductive system of camels may cause complications resulting in poor reproductive performance leading to infertility and consequent loss of productivity [5]. Thus, identification of reproductive diseases is important, especially when dealing with genetically superior [6].

In Ethiopia, production and productivity of *Camelus dromedarius* is facing multifaceted challenges where disease takes the major share among others [7]. For the diagnosis and management of reproductive performance

and to circumvent the reproductive disorders, histopathological investigations and isolations of bacteria from uterus are critical [8]. Despite the benefits of camels regarding their meat and milk, camel production is still not undertaken on a commercial scale [1]. There is scarcity of information about camels' reproductive disorders recorded, [9, 10]. Especially in countries like Ethiopia [11]. The objectives of this study were therefore, to identify pathological problems at gross and histological levels and to isolate bacteria from reproductive organs with abnormalities from dromedary camels slaughtered at Akaki abattoir, Ethiopia.

## MATERIALS AND METHODS

**Study Area and Population:** The study was conducted in Akaki municipal abattoir 20 KMs far from center of Addis Ababa where five to eight camels were slaughtered every day during the study period from October 2013 to May 2014. The limited number of animals slaughtered is due to the less demand of camel meat in Addis Ababa. Only the Somali people are using camel meat in Addis. Female camels bought from Afar and Borana were the study animals. Camels from Afar National Regional State are smaller in size and extremely hardy and Borana camels from Oromia National Regional State are of medium sized and both employed as pack, draught and dairy animals [12]. The reasons to take these camels for slaughter was not possible to determine as the owners are not in the study area.

**Study Design:** A cross sectional study design was conducted on 231 female camels examined for the pathological disorders and associated bacterial causes. Ages, physiological status and areas of origin were the variables of interest. Non probability sampling methods was used and all female camels slaughtered in the abattoir during every visit were examined for presence of any reproductive organ disorder. It was not possible to collect information related to infertility history since the merchants did not have any information; however, physical examination before slaughtering was conducted.

**Sample Collection and Transportation:** Everyday about 5-8 camels were slaughtered in the abattoir and 3-5 of them are female camels. Reproductive organs were removed from the animal and examined one by one for any gross pathological disease and disorders. Gross lesion examination was performed to see lesion distribution, contour, consistency, texture, shape, size and color.

Reproductive tract tissues with lesions were sampled for histopathology and bacteriological examination. The parts with lesions were cut to the size of 2-3 cm and put in the universal bottle containing 10% buffered formalin which stabilizes the tissues. The samples were transported to the National Animal Health Diagnostic and Investigation Centre (NAHDIC) Sebeta for processing.

For bacteriological examination, the surface of the tissue with the lesion was decontaminated by moderate hot application using flame and then collected separately in sterile universal bottle, labeled and transported to NAHDIC in icebox following Quinn *et al.* [13].

**Histopathology Procedures:** Tissue processing was conducted following the procedure described by Talukder [14] trimming, fixing in 10% buffered formalin, dehydrating, clearing and impregnating with molten paraffin wax. Then, the tissues were sectioned at 5µm thickness and stained with hematoxylin and eosin.

**Bacteriological Procedures:** All bacteriological procedures were performed according to Quinn *et al.* [13]. The sample tissue was minced with sterile scissors and forceps; inoculated to brain heart infusion broth and aerobically incubated at 37°C for 24hrs. After 24hrs, a loop-full of bacterial growth were streaked on 7% sheep blood agar and incubated aerobically at 37°C for another 24hrs. Then, the plates were checked for presence of growth and examined for colony morphology, size, shape and presence or absence of haemolysis. The colony was sub-cultured on blood and MacConkey agars. For primary identification catalase, oxidase, oxidation fermentation, motility tests and gram stain was conducted. Selective and differential media such as mannitol salt agar, Edwards medium, eosine methylene blue (EMB), xylose-lysine-desoxycholate (XLD) and salmonella shigella agar were used for the suspected samples from the primary test results. After 24hrs of incubation characteristic growth on selective medium was registered.

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

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

and pregnancy status was evaluated and only pregnancy status has significant difference (Figure 1). Association of gross lesion types with age, origin and pregnancy was also evaluated. Follicular cyst was observed to be five times more in Afar camels than in Borana camels (OR=4.86, 95%CI, 1.12 – 20.96, P=0.034). However, no significant difference was observed for other types of gross abnormalities (Table 2).

**RESULTS**

Reproductive organ abnormalities and lesions characterizations: Of the 231 female camels slaughtered in Akaki abattoir and examined for gross pathological disorders 46 (19.9%) has gross pathology in their reproductive organs (Table 1). Thirty (17.76%) of these camels come from Borana and 16 (25.8%) were from Afar. The occurrence of reproductive disorders by origin, age

**Ovarian Abnormalities of Dromedary Camels**

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

Table 1: Gross reproductive tract abnormalities with their respective numbers and percentages among dromedary camels slaughtered (N=231) during the study period in Akaki abattoir, Ethiopia

No.	Types of lesion	No. of camels with gross lesion	Percentage (%)
1	Follicular cysts	8	3.5
2	Ovarian hypoplasia	1	0.43
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	19.3

Table 2: Percentages and values of significance of gross reproductive tracts of *Camelus dromedarius* slaughtered at Akaki Abattoir, Ethiopia

Factors considered with their respective X <sup>2</sup> and P-value, for each abnormality	Types of reproductive tract abnormalities in dromedary camels							
	Follicular cysts	Endometrial lesions	Uterine cysts	infundibular cyst	Para ovarian cyst	Ovarian hypoplasia	Ovarian tumor	
Origin	Afar	5 (8%)	8 (12.9%)	1(1.6%)	0	1(1.6%)	1(1.6%)	0
	Borana	3 (1.7%)	23(13.6%)	0	1(0.59%)	1(0.59%)	0	2(1.18%)
	X <sup>2</sup>	5.37	0.02	*	*	*	*	*
	P-Value	0.021	0.89	2.7	1.0	0.47	0.27	1.0
Age (Years)	≤9	4 (3%)	20 (15.2%)	1(0.8%)	1(0.8%)	1(0.8%)	1(0.8%)	0
	>9	4(3.4%)	11 (11.1)	0	0	1(1%)	0	2(2%)
	X <sup>2</sup>	*	0.79	*	*	*	*	*
	P-value	0.73	0.37	1.0	1.0	1.0	1.0	1.83
Pregnancy status	Pregnant	0	0	0	0	0	0	0
	Non- pregnant	8(4%)	31(15.7%)	1(0.5%)	1(0.5%)	2(1%)	1(0.5%)	2(1%)
	X <sup>2</sup>	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 have no X<sup>2</sup> value

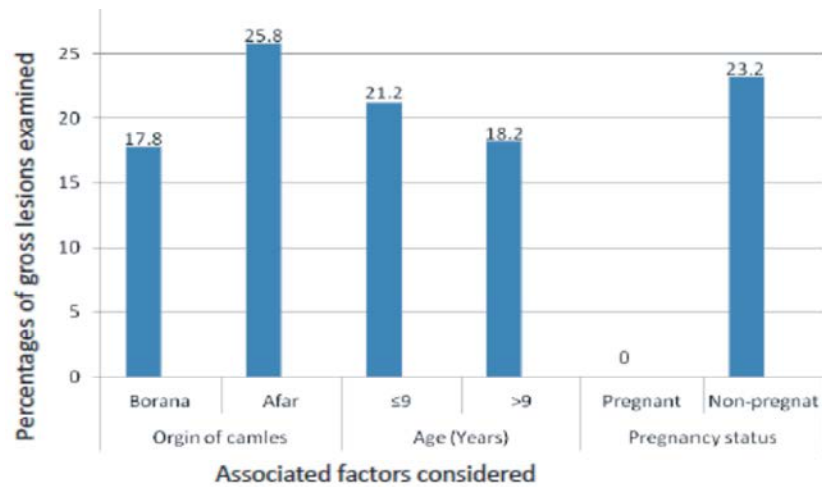


Fig. 1: Distribution of reproductive tract disorder associated with Origin, age and pregnancy status of dromedary camels slaughtered in Akaki abattoir, Ethiopia

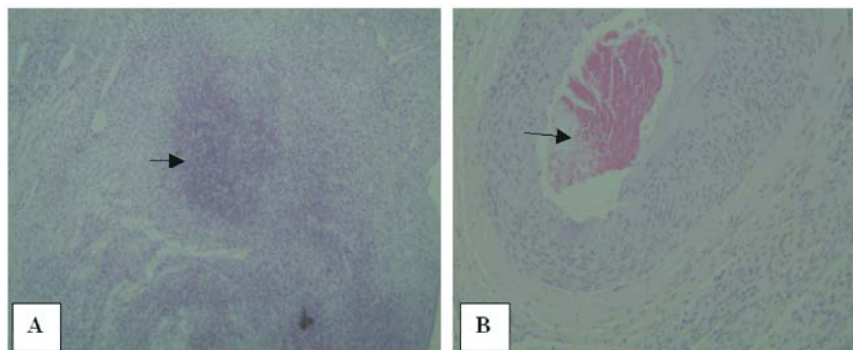


Fig. 2: Hematoxyline-Eosine (H and E) stain of atrophied ovary. (A) hypoplastic ovary showing aggregation of cells (arrow) in one area without follicle formation, (B) hypoplastic ovary with congested blood vessel (arrow) seen in the center of less developed follicle (40x)

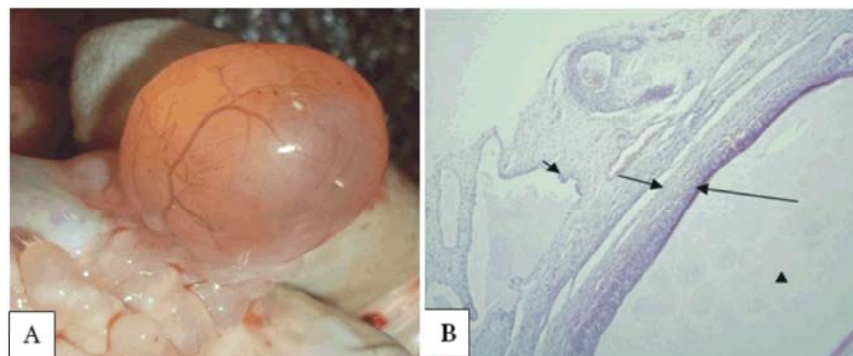


Fig. 3: Follicular cyst of dromedary camels. (A) gross and (B) histologic features of follicular cyst after (H and E stain) (arrow head = proteinaceous fluid, short arrow=theca externa and congested blood vessels, medium arrow=theca externa, long arrow= thin granulose cells) (40x)

Histopathologically the follicles were few in number, not organized; blood vessels were congested in the center of the follicles and instead of forming follicles of different types and cells were

simply aggregated in to focal area. Hyperplasia or excessive proliferation of stroma cells and fibroid connective tissue were also detected (Figure 2 A and B).

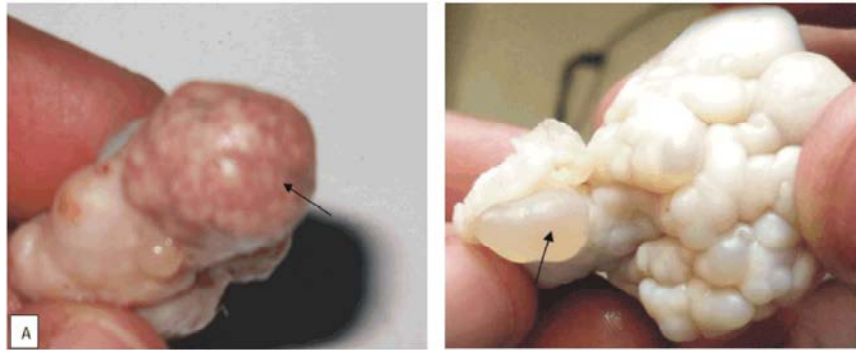


Fig. 4: Macroscopic features of ovarian tumor (A) and para-ovarian cyst (B) in dromedary camels

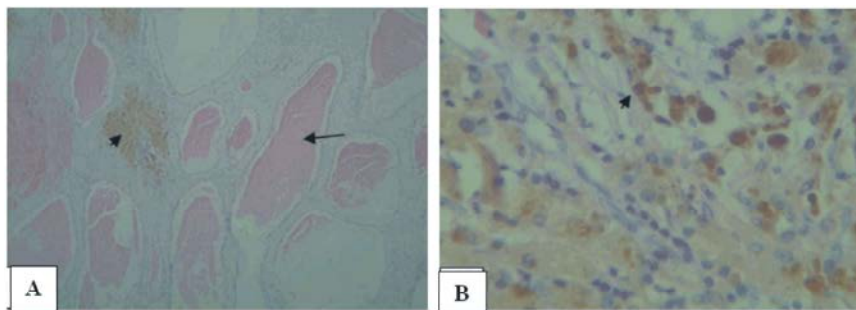


Fig. 5: Histopathology of hemangioma in dromedary camels after H and E stain

**Follicular Cysts in Dromedary Camels:** Follicular cysts were detected in 3.5% of camels examined. These cysts contained clear fluid, enclosed in thin layer of connective tissue and granulosa cells. These cysts were externally pale and well vascularized. The sizes of these cysts ranged between 23mm-36mm and all of them were on the left side (Figure 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 A and B).

**Ovarian Tumor and Para-ovarian Cyst:** Two of the non pregnant camels slaughtered during the study period showed unilateral ovarian tumors (hemangioma) on the left ovaries. Grossly they were well demarcated, firm in consistency, 1.1 mm X 0.8 mm in length and width, almost circular in shape and dark-red in color. The outer surfaces of the neoplasms were smooth with prominent vessels and blood oozed from the cut surfaces (Figure 4A). The remaining ovarian tissue was pale to gray and various numbers of corpus lutea and follicles were found in the ovaries. In one case two parovarian cysts were appeared simultaneously in the ovary and in other case only one cyst was found on one side of the ovary (Figure 4B).

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 5A and B).

Para-ovarian cysts are those cysts located near the ovary attached to the broad ligament and identified in two camels slaughtered. They were detected unilaterally only on the left sides of the ovary.

#### **Uterine Abnormalities and Lesion Characterization**

**Uterine and Infundibular Cysts:** Uterine serosal cyst was found in 1 (0.5%) of the examined uterus. This cyst which developed on the left side of the uteri contains clear fluid and measured about 10mm in size (Figure 6A). Infundibular cyst was a clear to serous fluid filled cyst which measured about 32mmx24mm of length and width. The wall of this cyst was externally thin, pale and clear-serous fluid oozed out during incision (Figure 6B).

**Endometrial Lesion:** Uterine hemorrhage and endometrial congestion were found with high incidence rate than other pathological cases accounting for 13.4%.

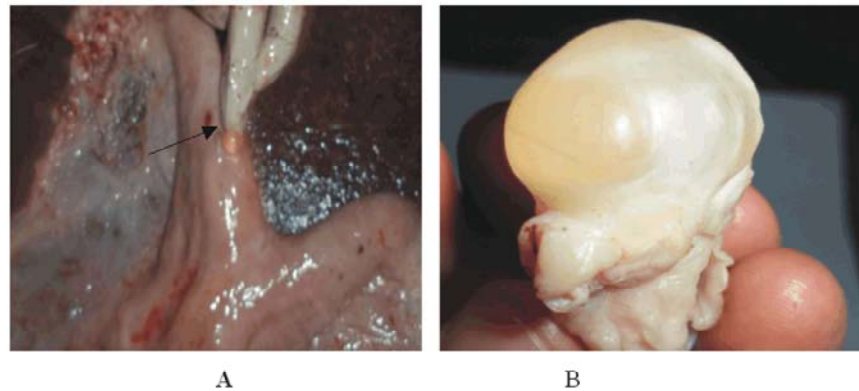


Fig. 6: Gross lesion appearances of uterine serosal cyst (A) and infundibular cyst (B) in dromedary camels

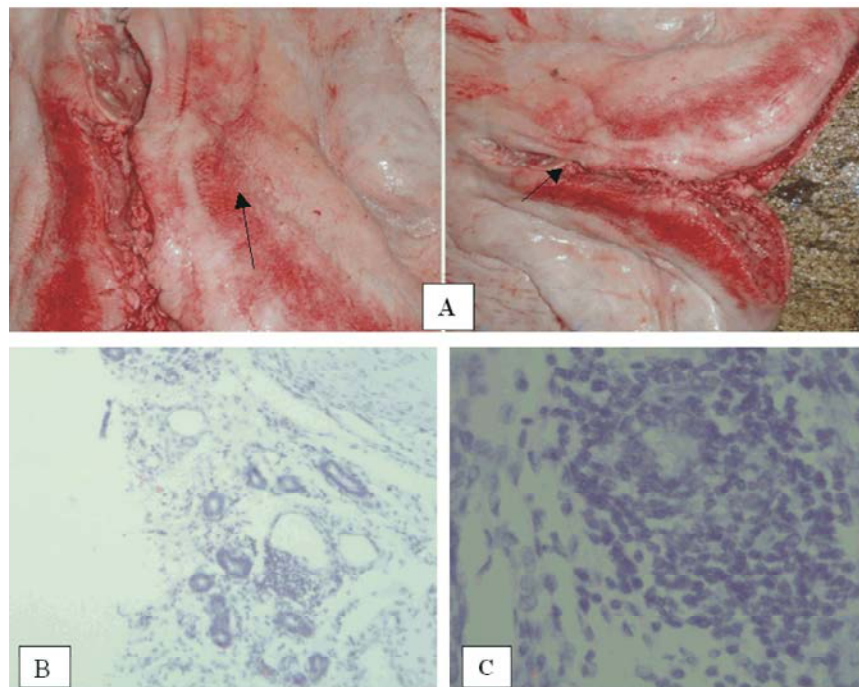


Fig. 7: Gross lesions and microscopic pathology of endometrium with hemorrhage and congestion in (A) and (B) respectively. Periglandular cuffing of the lymphocytes in endometrium(C) (40x) in dromedary camels

Of which 5.2% and 6% were acute and chronic endometritis respectively. Macroscopically, these uteri were congested in some parts of the endometrium and in other cases the congestion and pin point hemorrhages were distributed over the entire parts of the endometrium, serosal parts of the uterus, including the body and horns of the uteri, even in some cases extended to cervix and vagina (Figure 7A).

In acute endometritis gross congestion of the endometrium and enlarged (increased in width) was observed. Histologically hemorrhage and infiltration of the polymorphonuclear cells (neutrophils) in endometrial

layer of the uterus was detected (Figure 7B 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 and formation of edema in myometrium were detected (Figure 8A,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. Histologically calcification of the endometrium and infiltration of macrophages were clearly observed (Figure 8D).

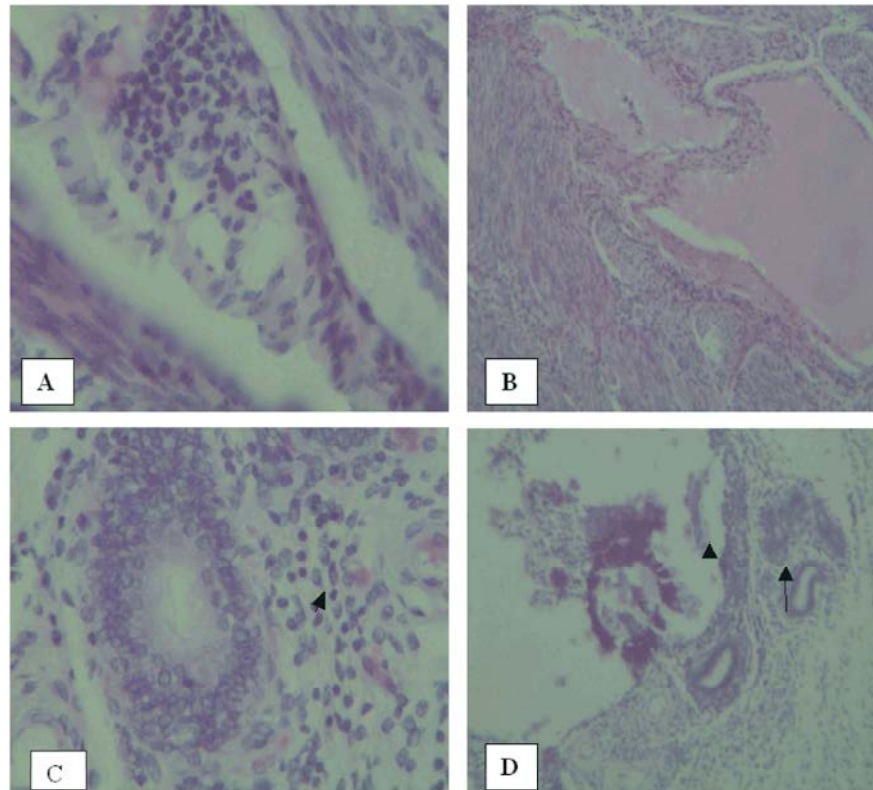


Fig. 8: Uterine problems in dromedary camels with neutrophil infiltration in the myometrium (A), edema in myometrium (B), hyperplasia of glandular cells in which cells are enlarged and pale in color (dot arrow) and infiltration by plasma cell (long arrow) (C), Calcified substance with in the endometrium and infiltration of the macrophages(D) after H and E stain (40x)

Table 3: 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 4: Association of bacterial detection with origin, age and pregnancy status of the slaughtered female dromedary camels at Akaki Abattoir

Risk factors	Categories	Number of examined camels	Number of positive camels	Percentage (%)	$\chi^2$ 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 endometritis	12	12	100	18.22	0.001
	Chronic endometritis	14	14	100		
	Simple congestion	5	0	0		

**Bacterial Isolation:** From 31 camels examined for endometritis, different types of bacteria were isolated in 26 (83%) camels and the main bacterial isolates were *Escherichia coli* and *staphylococcus* species (Table 3).

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 in bacterial isolates between age groups and origins of camels, but significant differences of bacterial isolation were seen among different types of lesions (Table 4).

## DISCUSSION

The results of this study demonstrated that reproductive organ diseases and disorders are important problems affecting the reproductive performance in female dromedary camels. The reproductive disorders were reported from different countries [5, 6, 15-18] though no such report from Ethiopia. Compared with previous reports, the current result was relatively with lower occurrence rate. Statistical significant difference ( $P < 0.05$ ) in the occurrence of the reproductive organs disorder between the pregnant and non pregnant camels was observed. It is obvious that to find abnormalities in pregnant animals is less likely.

Pathological lesion of endometrium was the common reproductive problem reported in previous studies. The present study also determined endometritis as a major reproductive organ abnormality followed by follicular cysts. However, the incidence of acute endometritis in the present study is lower than the reports in Saudi Arabia by Adel *et al.* [5]. The microscopic finding of the chronic endometritis was similar to the reports in Egypt by Shawky *et al.* [16]. A very large incidence of the endometritis (45.9%) was also reported in Saudi Arabia by Ali *et al.* [6]. These variations might arise from differences in management system and veterinary services as well as nutrition and physiological differences of the camels at the different study countries. Over breeding, postpartum complications and unsanitary gynecological manipulation were the possible contributing factors to uterine infection in dromedary camels [19]. Aggressive mating during the “wrong” phase of follicular developmental has been reported as a cause of severe uterine inflammation [2, 20]. Postpartum complications, unsanitary gynecological manipulations and errors including breeding with a young male, overuse of males and lack of verification of intromission during copulation have been reported as causes of endometritis [6].

The total incidence of the ovarian disorders in this study was 4.8% including ovarian hypoplasia, ovarian hemangioma and ovarian follicular cyst lower than the finding by Adel *et al.* [5] and by Shawky *et al.* [16] with 17.14% and 40% respectively. The differences could be attributed to nutritional, seasonal and genetics [5]. Individual differences in identifying the pathologies might also be sources of differences among different reports.

The statistically significant difference in occurrences of follicular cysts between Borana and Afar areas might be due to variation in breeding practices of camels from the two areas as camels are induced ovulators. Adel *et al.* [5] justified the probable variation in the occurrences of ovarian cysts to be due to variation in the heat stress. This supports the report of the present study as the two areas are different in their temperature. Ovulation failure is caused by inadequate LH release in response to copulation [21, 22] and this could also be one of the factors for higher rate of incidences of follicular cysts among dromedary camels. The finding of ovarian follicular cysts in the present study was in agreement with the report in the eastern region of the Saudi Arabia [5] and slightly lower than in Kalyoubia, Egypt [16] and higher than report from Iran [15]. The maximum size of follicular cyst reported by in Egypt [5] was higher than reported in this study. The variation in the size of the follicular cyst could be due to age differences of the cysts.

The bilateral ovarian hypoplasia is comparable to previous report of Shawky *et al.* [16] but lower than the reports by Adel *et al.* [5]. The incidence of ovarian hemangioma in this study is in line with Shawky *et al.* [16] but higher than Adel *et al.* [5]. The effect and causes of ovarian tumor needs further study. The para-ovarian cyst report in the current study is lower than the reports by Nourani *et al.* [15] and Shawky *et al.* [16] but higher than report of Adel *et al.* [5]. The size of the cysts presented in this report was lower than previous reports of Shawky *et al.* [16]. The infundibular cyst previously reported in Saudi Arabia by Mahmoud *et al.* [17] and Adel *et al.* [5] were higher than the current report. The size of the infundibular cysts reported in this study is also lower than the size of the report from Saudi Arabia [23]. In all of the reports, the cause of infundibular cysts is not clear.

The presence of cysts on the uterine serosa and broad ligament was in agreement with the finding of Skidmore and Adams [21]. Calcification in the endometrium is the first report in dromedary camels. Calcium salts, usually in the form of phosphates or carbonates, may be deposited (calcium buildup) in dead, dying, or normal tissue [24].



The 45 bacteria of 7 genera isolated from the endometritis in this study, is in line with the reports of Mshelia *et al.* [18]. The bacteria colonizing the reproductive tract of the female *Camelus dromedarius* have been incriminated as major causes of reproductive disorders in this species [18]. These are regarded as the most important cause of infertility in domestic species. The reports of *Escherichia coli* from chronic endometritis and isolation of the *Proteus vulgaris* in acute endometritis in this study was similar with the report of Adel *et al.* [5]. Similarly the isolation of *Staphylococcus species*, *Corynebacterium species* and *Salmonella species* from endometritis are in line with the reports of Donnald and Zachary [24] from Sudan. Isolation of the *streptococcus*, *Enterobacter aerogenosa* and *Salmonella species* from endometritis is in line with reports of Tibary and Anouassi [25] and Abdel-Aziz and Nasser [1] and these evidences implicate bacterial infection as a cause of endometritis [18, 26]. The role of these bacteria as a cause of endometritis and infertility in dromedary camels needs further investigations.

In conclusion, macroscopically, different pathological abnormalities of reproductive organs of female camels were recorded and affected organs were processed in laboratory for histopathology and bacterial isolation. Endometritis was the major reproductive organ problem recorded during the study period followed by ovarian cysts. Seven bacterial genera were isolated and identified from endometritic cases, among which *E. coli* and *staphylococcus* species were the major ones. The role of each reproductive problem incriminated as causes of reproductive failures in this species needs further investigation. The socioeconomic effects of these problems in the pastoral areas need to be specified. Collaborative researches are critically important to improve the productivity of *Camelus dromedarius* and devise proper intervention mechanisms for the identified problems.

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