



Histopathological observations of farm animal mastitis in Al Muthanna governorate abattoir

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Abstract

Mastitis is an inflammation of the mammary gland that causes economic losses in milk production and earlier animal culling. Mastitis is associated with multiple changes, such as, physical, chemical, and microbial in milk, and various histopathological features in the udder. This study intended to investigate the histopathological findings of mastitis

in abattoir in the farm animals, including cows, buffaloes, ewes, and she-goats, slaughtered in the Al-Samawa abattoir/Al Muthanna governorate. Fifty-one mammary gland tissues were collected from various slaughtered animals at Al-Samawa abattoir for two months (December 2021 -February 2022). Each udder sample was divided into small pieces and immersed in 10% neutral buffered formalin solution for histopathological processing and evaluation. The total samples of the current study were 51, which comprised of 9 cows, 10 buffaloes, 14 ewes, and 18 she-goats. The number of pregnant animals was 5, 7, 9 and 12 for cows, buffaloes, ewes, and she-goats respectively, while the number of non-pregnant cows, buffaloes, ewes, and she-goats were 4, 3, 5 and 6 respectively. The percentage of abattoir mastitis and normal animals were 100%, and 0.00 %, respectively. Degeneration, necrosis of the udder alveoli, and infiltration of mononuclear and polymorphonuclear cells were the most prominent histopathological features. The proliferation of fibrous connective tissue was also common in the interstitial glandular tissue. In conclusion, the current study approved that histopathological investigation offers a good tool for the diagnosis of mastitis. The authors suggest considering and validating udder biopsy and histopathological examinations to diagnose of mastitis in animals suspected with udder damage, before culling. It also helps to make a decision to treat and follow up the infected animals. The authors recommend future patho-epidemiological study on mastitis, to determine its actual occurrence in animals, in Iraq.

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Introduction

Mastitis in farm animals indicates inflammation of the mammary glands, causing physical, chemical, microbial changes, and an increase in somatic cells in the milk. It is



characterized mainly by pathological changes in the udder tissue. Mastitis is a multifactorial disease due to environmental predisposing factors and microbial infection in the teat canal and mammary tissue. The pathogenesis of mastitis is a complex process (Pankaj *et al.*, 2020). The predisposing factors contributing to mastitis are innate and adaptive immunity interactions, physical damage to the milk sphincter and factors associated with mechanical milking (Schlafer and Foster, 2016). Mastitis causes a significant impact on dairy herds and can be caused by numerous agents, among which the *Staphylococcus* genus is the most frequently isolated (Ajose *et al.*, 2022; Sztachańska *et al.*, 2016; Contreras and Rodríguez, 2011). In early lactation, inflammation of mammary parenchyma results in long-term production loss and possibilities of premature culling from the herd. Infection of the mammary gland with various pathogenic agents may result in liberating harmful toxins in the udder (Ajose *et al.*, 2022; Yousaf *et al.*, 2010). In this case the mammary lesions fluctuate from increased milk leukocyte counts, with no gross alterations in milk, to increased vascular permeability, severe toxemia, and developing fibrosis (Sinha *et al.*, 2022; Oviedo-Boyso *et al.*, 2007; Ibrahim *et al.*, 2011). Several types of leukocytes, such as, neutrophils, macrophages, and lymphocytes, accompanied with somatic cells are important features of mastitis (Adkins and Middleton, 2011; Abera *et al.*, 2010). In the early stages of mastitis, the leukocytes play an important role in the establishment of intra-mammary infection. The marked infiltration of leukocytes during mastitis is considered to be damaging to the developing mammary parenchyma tissue (Adkins and Middleton, 2011; Abera *et al.*, 2010; Nickerson, 2009), eventually causing milk loss (Derakhshani *et al.*, 2018; Piepers *et al.*, 2009). The normal lactating mammary gland is characterized by major changes comprising of an increase in the number of alveoli, alveolar lumen, and decrease in connective tissue. The characterization of udder parenchyma shows the influence of probable milk production on the development and secretory cell differentiation (Zigo *et al.*, 2021; Patel *et al.*, 2007; Holland and Holland, 2005).

The incidence of clinical mastitis ranges between 15% and 25%, although in some herds it can be higher than 45%². Despite the modern methods established for the control of bovine mastitis, it continues to be the main disease causing economic losses in dairy production, animal suffering, negative effects on milk quality, and reduction in product hygiene (Ajose *et al.*, 2022; Sztachańska *et al.*, 2016; Contreras and Rodríguez, 2011). It is impossible to accurately quantify the total loss in cost due to mastitis, it varies over time and depends on the herd conditions. The decrease in milk production represents approximately 70% of the total cost of mastitis (Ruegg and Reinemann, 2002; Oviedo-Boyso *et al.*, 2007). In the United States, the cost of mastitis has been estimated at \$200/cow/year (Schlafer and Foster, 2016) in clinical evaluation, and could be diagnosed by laboratory and field tests, such as the California Mastitis Test (CMT) (Chagunda *et al.*, 2006; Zhao and Lacasse, 2008; Watban *et al.*, 2021). This test is was easy and fast to predict the somatic cell count in milk of cows with affected mammary quarters (AL-Edany *et al.*, 2012).

The pathogenicity of microorganisms and their ability to invade tissues, as well as, the resistance of the mammary glands, are related to the occurrence and type of mastitis and its symptoms. These factors determine the severity of symptoms, which can range from increased somatic cell counts without macroscopic changes in milk to progressive fibrosis or the occurrence of severe toxemia (AL-Edany *et al.*, 2012; Bhutto *et al.*, 2012). Histopathology is significant in evaluating tissue damage and loss of structural integrity,



depending on the severity and duration of the condition. In chronic mastitis the udder reveals inflammation, fibrosis, increased cells, edema, and atrophy of the udder tissue, whereas, abscesses and gangrene appear in severe cases (Ruegg and Reinemann, 2002; Chagunda *et al.*, 2006; Zhao and Lacasse, 2008; Watban *et al.*, 2021; AL-Edany *et al.*, 2012; Bhutto *et al.*, 2012; Benites *et al.*, 2002). A review of literature has revealed massive reports on the bacteriology, diagnoses, and epidemiology of mastitis, however, there are very few publications relating to the basic histopathological knowledge and changes in mastitis, in farm animals. Therefore, this study intends to describe the histopathological findings in mastitis, in the mammary glands of farm animals, including cows, buffaloes, ewes, and goats that are slaughtered in the Al-Muthanna abattoir.

Materials and Methods

Ethical statement

The current study was approved by the Research Ethical Committee / College of Veterinary Medicine/ Al Muthanna University 2021–2022. Furthermore, all samples from the animals were collected by veterinarian according to the international standard, considering animal welfare and ethics.

Samples

A total of 51 mammary tissues from different animals including cattle, buffaloes, ewes, and she-goats, were collected from the Al-Samawa abattoir, during a period of two months, extending from December 2021 to February 2022. Each udder sample was divided into small pieces and immersed in 10% neutral buffered formalin solution. All samples were sent for histopathology to the laboratory, for routine tissue processing. Eventually, the samples were embedded in paraffin and sectioned into 5 μ m thickness. Then the tissue sections were stained with Hematoxylin and Eosin. For evaluation of the histopathological changes, all sections were examined under a light microscope, connected with an image analyzer and camera (Leica) for capturing the images.

Results

In this study, 51 udder samples, including 9 cows, 10 buffaloes, 14 ewes, and 18 she-goats were collected from Al-Samawa abattoir. The number of pregnant animals were 5, 7, 9, and 12 for cows, buffaloes, ewes, and she-goats respectively, while it was 4, 3, 5, and 6 for non-pregnant cows, buffaloes, ewes and, she-goats respectively. The total percentage of the abattoir histopathological mastitis in the collection samples from all species was 100%, while the percentage of normal animals was 0.00 %. The percentages of histopathological mastitis for each species were 100%, 100%, 100%, and 100% for cows, buffaloes, ewes, and she-goats respectively (Table. 1, Figures. 1 A and B).



Table.1: Shows the number and specification of the slaughtered animals used in this study

Species	Total	P	NP	HPL	NHPL
cow	9	5	4	9	0
Buffaloes	10	7	3	10	0
ewes	14	9	5	14	0
She-goats	18	12	6	18	0
Total	51	33	19	51	0

P= Pregnant; NP= Non- Pregnant; HPL= Histopathological Lesions; NHPL= No Histopathological Lesion

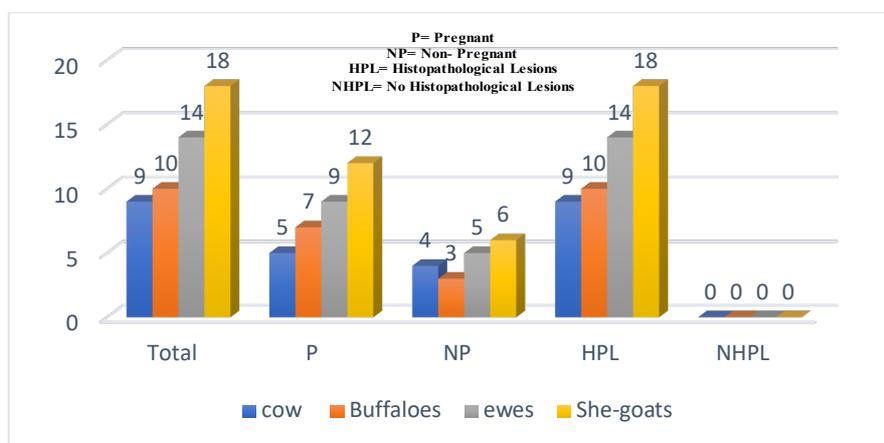


Figure. 1: Shows the number and distribution of samples

Macroscopic investigation

Grossly, all udders of the slaughtered animals appeared normal during examination at slaughtering. (Figures. 2, 3, 4, 5).



Figure. 2 : Shows normal appearance of the udder of the slaughtered cow

Figure. 3 : Shows the lactating mammary glands from buffaloes.



Figure. 4 : Shows lactating mammary glands from she-goat

Figure. 5 : Shows normal appearance of the udder of the slaughtered ewe

Microscopic investigation

Numerous histological lesions were seen in the udder sections prepared from different species of animals. The lesions revealed various stages of mastitis, from acute to chronic, and some cases showed granulomatous lesions. The observed lesions varied in different species; therefore, the results of the histopathologic changes in the current study are presented according to the species of the animals.

Cow

Nine udder samples were collected from the cows during this study. These were five samples from pregnant and four from non-pregnant cows, respectively. The histopathological lesions (HPL) were obvious in all samples (9 out of 9) at 100%. The examined udder sections revealed various HPL between different examined sections and between different areas in each section. All bovine udder sections revealed scattered lesions in the interlobular and mammary alveoli. The milk alveoli showed that variable sizes dispersed degenerative necrotic foci, containing clusters of bacterial debris, surrounded by necrotic alveolar epithelial cells. The necrotic foci were fused and formed large foci. Some udder sections revealed a different degree of hyperemia in parenchymal areas. Moreover, other areas showed infiltration of neutrophils and mononuclear cells accompanied by edematous loosening. The udder sections also showed various lesions in the interlobular duct's epithelium lining, which was replaced by necrotic debris mixed with bacterial clumps. Some sections revealed degeneration and necrosis of mammary gland lobules. The affected areas also revealed fibrinous necrosis, with fibrinous thrombi in their interlobular stroma (Figure. 6. A, B, C, D).

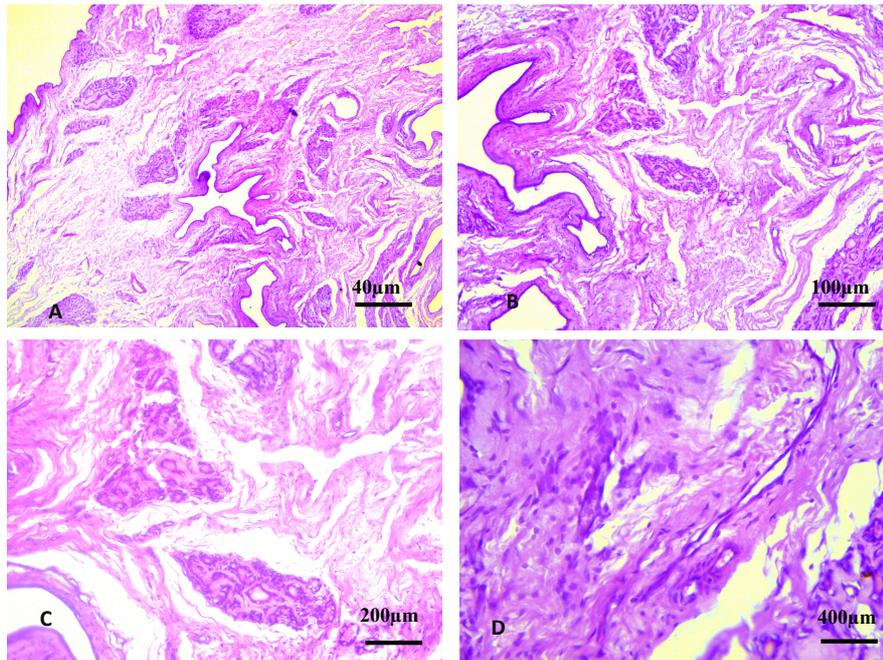


Figure. 6 A, B, C, D: Section of cow's udder shows : necrosis of in the interlobular and in the mammary alveoli and fibrinous thrombi, Necrosis lesion distributed in the alveoli and interlobular duct. Hyperaemia and accumulation of inflammatory cells, infiltration of mononuclear cells and necrosis of the necrosis of intralobular ducts and mammary alveoli. A (X4); B: (X20); C (X20) ; D. (X40).

She-Goat

Eighteen she-goat udder samples were collected during this study. These were 12 from pregnant and 6 from non-pregnant goats, respectively. The HPL were seen in all samples (18 out of 18) at 100%. The examined sections revealed various HPL between different sections and between various areas in each section. However, all the she-goats' udder sections revealed numerous histopathological features with typical inflammatory responses, severe replacement of fibrous connective tissue, and atrophy of glandular parenchyma. Red blood corpuscles (RBCs) were seen, along with fibrotic proliferation and cellular inflammatory infiltration, in the secretory alveoli, which contributed to the animal's active immune response against infections. The congestion and hemorrhages were more evident in the glandular lumen filled with erythrocytes and fibrotic cell proliferation. Moreover, the milk's alveoli showed severe damage, characterized by sloughing and exfoliation of the epithelial lining and alveolar wall necrosis. The swollen and cystic alveoli were also apparent in some she-goat cases. Most she-goat udder sections revealed evidence consistent with chronic mastitis, comprising of exposure of the alveolar basement membrane due to necrosis of the alveolar epithelium. Additionally, congested acini and proliferation of fibrous tissue were observed in both the interlobular and interacting areas. Degenerative lesions were evident in the acinar epithelial cells. Few sections revealed indurations of the udder parenchyma that were accompanied with mild interstitial necrosis and a prominent reduction in the acini. Most cases clearly showed the infiltration of substantial mononuclear cells comprising of

lymphocytes, histiocytes, and a few polymorphonuclear (PMNs) cells (Figure. 7 A, B, C, D).

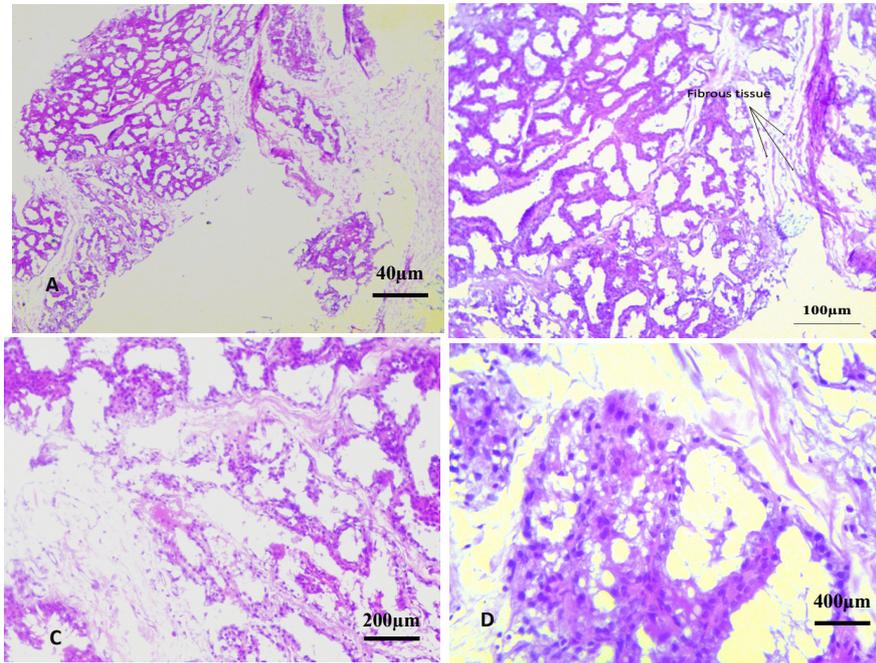


Figure. 7 A, B, C, D: Section of she-goat's udder shows : Disruption of the udder alveoli , Necrosis lesion appears along the alveoli and interlobular duct with obvious fibrosis , Sloughed cell and infiltration of inflammatory cells, Fibrosis in the alveoli and intratubular accompanied with Infiltrations of polymorphonuclear cells of the necrosis of intralobular ducts and mammary alveoli. A.(X4); B.(X20); C: (X20); (X40)

Buffaloes

Ten buffalo udder samples were collected during this study. These samples were seven and three pregnant and three from non-pregnant buffaloes, respectively. The HPLs were seen in all samples (18 out of 18) at 100%. Diverse histopathological lesions were seen in the udder sections of buffaloes. Furthermore, both acute and chronic histopathological lesions were observed. The observed acute histopathological lesions were an infiltration of various inflammatory cells accompanied by damage of the acinar epithelium. In contrast, the chronic lesions revealed various degrees of focal or diffuse fibrous tissue proliferation. All udder sections of non-pregnant buffaloes revealed desquamation of the acini luminal epithelium and vascular degeneration. Moreover, the caseated milk was filled with acini, accompanied by an accumulation of fibrin network. Both interstitial connective tissue and mammary acini were profoundly infiltrated with neutrophils, macrophages, a few plasma cells, and lymphocytes. Few areas revealed focal edema and hemorrhage between the interstitial connective tissue and alveoli (Figure. 8 A, B). Only few sections from non-pregnant buffaloes revealed a chronic focal lesion characterized by the existence of non-functional connective tissue and acini. Moreover, different inflammatory cells that invaded the tissue predominantly were mononuclear cell macrophages and lymphocytes (Figure. 8.C and D). Most udder sections from pregnant buffaloes revealed chronic histopathological lesions comprising

of hyperplasia of the duct and massive proliferation of interstitial connective tissue and aggregation of inflammatory cells, mainly mononuclear cells (Figure. 8.E).

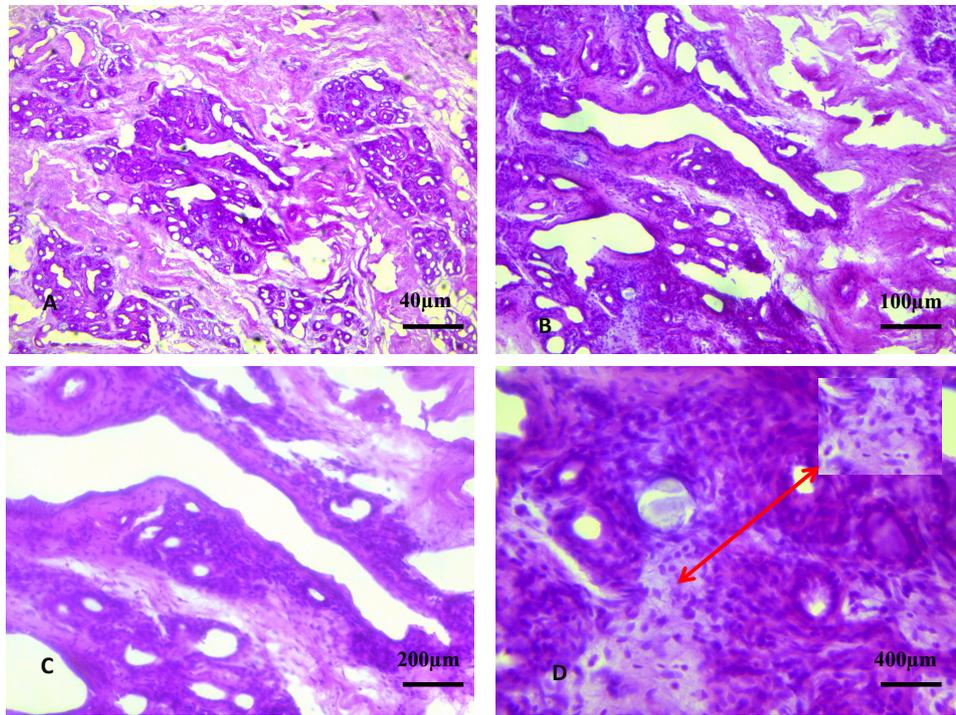


Figure. 8 A, B, C, D: Sections of buffaloes 's udder shows : inflamed udder alveoli with caseated milk Accumulation of inflammatory cells and necrosis lesion , two headed red arrow , the aggregation of MNCs, Infiltrations of polymorphonuclear cells and Fibrosis in the alveoli and intratubular. A:(X4); B. (X10); C(X20); D (X40)

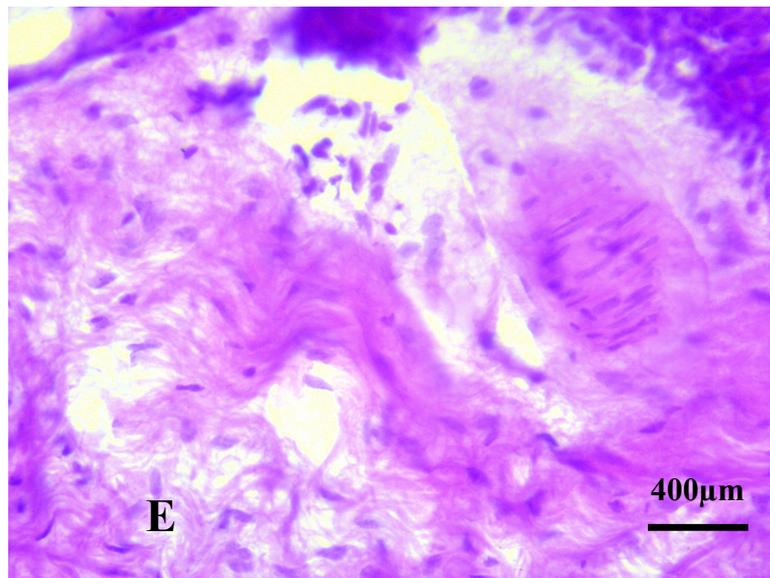


Figure. 8 E: Section of buffaloes 's udder shows aggregation of inflammatory cells and proliferation of connective tissue. (X40).

Ewes

Udder samples of 14 ewes were collected for this study. These samples were, nine from pregnant and three from non-pregnant buffaloes, respectively. The HPLs were seen in all samples (14 out of 14) at 100%. Both acute and chronic histopathological lesions were seen. Acute histopathological lesions were mostly seen in udder sections collected from non-pregnant ewes, accompanied by massive infiltration of polymorphonuclear cells, accompanied by damaged acini and accumulation of cellular debris and edema of interstitial connective tissue (Figure. 9 A and B). Chronic histopathological lesions were seen in most udder sections from pregnant ewes. The chronic lesion involved the fibrous proliferation of the duct accompanied by a proliferation of interstitial connective tissue and accumulation of mononuclear cells. Both diffuse and focal fibrous lesions were seen in most sections (Figure. 9 C and D).

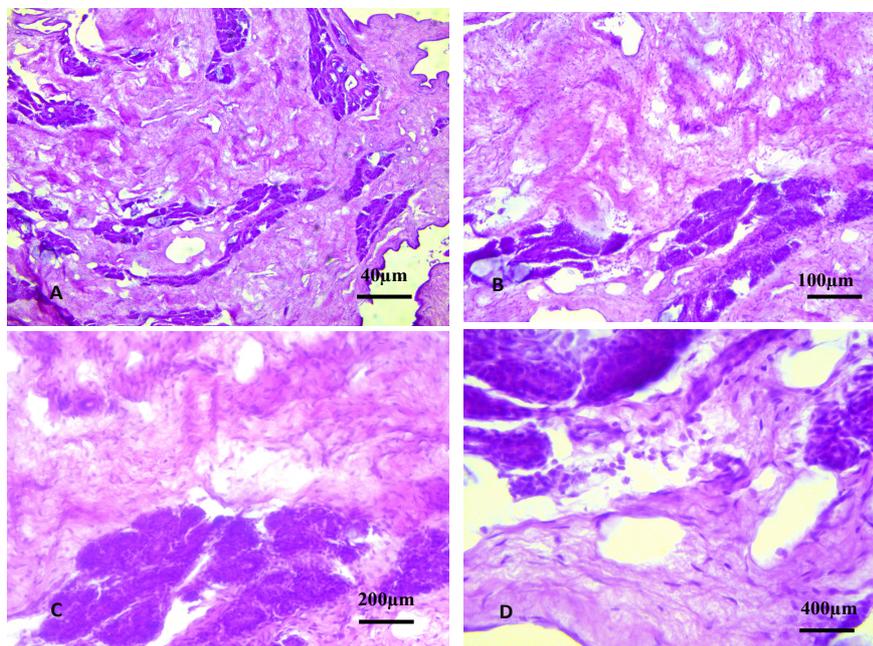


Figure 9. A.B.C.D: Section of ewe 's udder shows : damaged udder alveoli and sloughing of epithelial lining, accumulation of inflammatory cells and necrosis lesion, proliferation of fibrous tissue and infiltration of inflammatory cells with alveolar and intratubular fibrosis. A (X4); B. (X10) ; C. (X20); D: (X40)

Discussion

Mastitis of the farm animals is one of the crucial causes of the early culling of reproductive females from the herd. It is ranked as the number one disease in dairy animals in the field survey of major livestock diseases (Derakhshani *et al.*, 2018; Zigo *et al.*, 2021; Hussain *et al.*, 2012). Mastitis is a multifactorial disease that occurs due to the interaction between the host, environmental factors, and microbial agents. The types and severity of developing mastitis depend on the extent of inflammation, nature of the causative agents, duration of the disease, age of the animals, immunological status, and the state of lactation. Mastitis is associated with multiple changes involving the physical,

chemical, and microbial changes; increasing the somatic cell counting in milk content and pathological changes in the mammary gland tissue. Diagnosis of mastitis depends on different routine screening tests, which play an important role in the early diagnosis of clinical mastitis. However, in subclinical mastitis, there is neither a visual abnormality in the mammary gland nor in the milk, which can be used to recognize the disease. Therefore, histopathological changes are used as one of the diagnostic tests (Mayun *et al.*, 2008; Erskine, 2020).

In the current study, a histopathological examination was used to diagnose the occurrence of mastitis in farm animals slaughtered at the abattoir. According to the histopathological features, mastitis was observed in 51 out of 51 udder samples at 100%. Additionally, the percentages of abattoir histopathological mastitis were 100%, 100%, 100%, and 100% for cows, buffaloes, ewes, and she-goat respectively. This high percentage of mastitis may be displayed by the culling of females by farmers. The current study has reported higher percentages of abattoir mastitis than the percentages reported by previous Erskine, (2020). Malashko and Bashura, (2012), investigated the histological changes of the subclinical mastitis in the cow udder. They found that 9%, 4% to 13.7% of the udder parenchyma showed pathological changes without any obvious clinical signs, which could cause huge expenditures and losses. Moreover, the study also represented features of subclinical mastitis with difficulty in clinical diagnosis, but it has been proven in histopathological investigations. The study results also acknowledged that subclinical mastitis defeated all udder structural functional elements, including alveolus and interlobular ducts. The researcher also reported changes on the basis of glands, such as, 21.1%–33.9%, in udder thickness - 25.6%–40.4%, in the field of the milk cistern - 15.2%–35.4% of the entire lobule volume. It corresponded to 21%–33.5% of losses of all secreted apparatus of lobules, that is, 9.4%–13.7% of the entire mammary gland parenchyma is equivalent.

The current study reported histopathological features in the udder samples collected from cows, buffaloes, ewes, she-goats, and a she-camel. The reported histopathological changes were acute and chronic, accompanied by infiltration of different inflammatory cells and various degrees of proliferation of fibrous tissue. These results are compatible with the results reported previously by other

Researchers (Hussain *et al.*, 2012; Malashko and Bashura, 2012; Ebtsam *et al.*, 2020; Abba *et al.*, 2014; Ibrahim *et al.*, 2018; Hasan *et al.*, 2020). The histopathological changes of mastitis in the she-goats were reported by (Ebtsam *et al.*, 2020) using a surgical biopsy technique for the collection of udder samples from the animal. They described different characteristic features of chronic, diffused interstitial mastitis lesions matching subclinical mastitis. Furthermore, they concluded that the histopathological technique is a good tool for confirming the diagnosis of mastitis. The results of the current study reported the various histopathological changes in the buffalo udder samples, and these results agree with those of the previous researchers (Brunella *et al.*, 2019; Abeer *et al.*, 2008). However, Abeer and Hanaa, (2008), documented the microscopical features of acute and chronic mycotic mastitis in buffaloes, characterized by focal and diffuse granulomatous lesions. Moreover, the current study results also reported on the histopathological lesions of bovine mastitis, and these results were compatible with the observations reported previously (Bianchi *et al.*, 2019; Alaa *et al.*, 2019). However, Alaa *et al.* (2019) reported various bovine histopathological lesions (acute and chronic) that included necrotizing in mammary gland tissues. The current study results also reported the histopathological lesions of mastitis in small ruminants



(ewes and and she-goats), which agreed with the features previously reported by another researcher (Alaa *et al.*, 2018; Mohanty *et al.*, 2019). Those researchers described histopathological lesions, including infiltration of inflammatory cells, replacement of fibrous connective tissue, and congestion and hemorrhages.

In conclusions, this study acknowledged the significance of mastitis as a disease of farm animals that could cause damage to the udder function, earlier culling of animals, with risk to public health. The results of this study reported the occurrence of mastitis in different farm animals, including cow, buffaloes, she-goats, and ewes that were slaughtered in the Al-Muthhanna abattoir, according to the histopathological features. Both acute and chronic mastitis were reported in all these animals except the she-camel. The percentage of abattoir mastitis was 98.076%, which was higher than that reported in other studies in Iraq and elsewhere in the world. The most prominent histopathological features were degeneration and necrosis of the udder alveoli and acini, infiltration of both mononuclear and polynuclear inflammatory cells, and proliferation of fibrous connective tissue in the interstitial glandular tissue. The current study acknowledged that histopathological investigation is a good tool for diagnosing mastitis. Consequently, the authors suggested considering and validating udder biopsy and histopathological examinations, as tools for diagnosing mastitis in animals suspected of permanent damage, before culling. It also helps in treating the infected animals and assisting veterinarians to follow up on the affected animals. The author recommends another future study to focus on the pathology of mastitis in different species of animals, to determine the actual occurrence of abattoir mastitis.

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Ethics statement: The study was approved by animal ethical committee /College of veterinary Medicine /Al Muthanna University.

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Conflict of interest

The authors declare no conflict of interest in publishing this article.

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