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Histopathological and histochemical study of mastitis in sheep

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Abstract

Ovine mastitis is considered one of the problems that cause economic losses through its impact on milk production, which is unfit for human consumption. The current study aimed to shed light on mastitis in sheep in different areas of Mosul city. The results of the recent study showed the presence of mammary macroscopic and microscopic changes. Macroscopic changes were represented by the large size of the udder and changes in the color and texture, as well as clarity of sloughing and desquamation of the skin with clear exudation, especially in advanced cases. On the other hand, other samples showed the presence of hard nodules on the udders. In contrast, the histological changes represented by degenerative and necrotic changes 3.5%, and 21.4% of the sample appeared to suffer from liquefactive necrosis and abscesses infiltrated with inflammatory cells, 25% of the samples infected with granulomatous mastitis (nodular mastitis), other models showed that the udder infected with atrophy and metaplasia in the rate of 21%, thickening of the blood vessel wall with clot represented 14.2% and the deposition of calcium salt was 14.2%. The current study concludes that mastitis in sheep is widespread in sheep raised in different areas of Mosul city.

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Introduction

Mastitis is one of the most severe diseases that affect the industry of dairy animals. It is the primary cause for both low yield and poor quality of milk (1,2). It also has great importance for the dairy industry, particularly in developing countries, because it has high economic and social impacts. Moreover, it leads to premature animal culling and cost of treatment, also has diverse effects on the welfare of affected animals and causes decreased milk production (3), leading to reduced growth rates and increased mortality in lambs (4). Mastitis is a mammary gland parenchyma infection typically caused by bacteria, fungi, and viruses (5) that lead to physical, chemical, and bacteriological milk changes and lead to pathological changes in glandular tissue (6). Mastitis can be divided into two clinical and subclinical mastitis (7,8) clinical mastitis is two types: clinical mastitis characterized by obvious signs of animal disease, such as irregular secretion, hot swollen quarter or whole udder, and ewes may be suffered from rapid plus, loss of appetite, dehydration and depression in severe cases (9) while the subclinical mastitis, which has no visible sign (10,11) but causes an increase in milk somatic cell count and bacteria detection (12) microorganism play critical roles in the incidences of ewes mastitis especially *Staphylococcus* spp which consider the most causative agent (13).This study amid to determine the effects of mastitis on sheep in a different area of Mosul city.

Materials and methods

Ethical Approve

The experimental work was approved by ethical committee for animal extermination of the veterinary faculty of university of Mosul based on department scientific meeting on December the 15th 2019.

Collection of samples

Clinical examination and manual palpation were performed for 100 ewes from Veterinary Teaching Hospital, Wana district, Cockgley district, and Mushairefa district, from March to September 2019. The sample is from ewes suffering from clinical mastitis, representing 28 cases with clear signs of udder abnormalities.

Histopathological examination

Specimens were taken from diverse parts of the affected udders, fixed in 10% neutral buffer formalin and regularly processed embedded in paraffin wax, sectioned in 5-6 µm thickness stained with Harris' hematoxylin and eosin stain (14-16), Masson's and Mallory's trichrome stain methods were used for better clarification of the histopathological lesion.

Results

Prevalence of sheep mastitis

The current work results showed that the prevalence of sheep mastitis was 7% (Table 1).

Table 1: Showed the type of Histopathological lesion, percentage, and the number of infected ewes with mastitis.

Lesions	% Infected (no)
Degenerative changes	3.5% (1)
Liquefactive necrosis and abscess	21.4% (6)
Nodular mastitis	25% (7)
Atrophy and metaplasia	21.4% (6)
Thickening of the blood vessels wall	14.2% (4)
Deposition of calcium salt	14.2% (4)

Pathological Finding

The gross examination of clinically affected udders showed changes in the size, shape, color, and texture, including enlargement in one half of the udder or both discolorations, sloughing of skin and exudation, especially in the advanced cases, in some cases, the texture of the udder is soft. It contains fluid, while other infected ewes showed hard nodules (Figure 1).

Microscopic lesions finding

The percentage of the microscopic examination of tissue sections taken from mammary gland infected with mastitis equivalent is 30 % of specimens, which showed distortion in the shape and size of mammary alveoli, vacuolar degeneration with coagulative necrosis, sloughing of epithelial cell lining the mammary alveoli. Also, there is an accumulation of serous, proteinous substances in the lumen of the mammary alveoli; about 15% of collected specimens showed histological changes characterized by a multifocal area of liquefactive necrosis (micro abscess) with infiltration of inflammatory cells. The granulomatous reaction was equivalent to 20% in the alveolar parenchyma, which showed atrophy of alveolar tissue with infiltration of mononuclear inflammatory cells such as macrophage, lymphocytes, plasma cell, fibroblast, and giant cell. Others showed replacement of fibrous tissue instead of alveoli, diffused fibrosis which is equivalent to 20% of the affected cases. Atrophy of the alveoli and metaplasia of the glandular structure of mammary alveoli are equal to 15 %. Additionally, another section showed deposition of calcium salt in the lumen of some mammary alveoli. At the same time, other sections showed thickening in the interstitial tissue due to deposition of collagen fibrous, in addition to thickening of blood vessels wall and thrombus (Figures 2-5).

Figure 1: photograph of sheep udder (A) showed rigidity of udders and contained nodules. (B) pus and swelling of the udders. (C) congestion and hemorrhage inside the tissue.

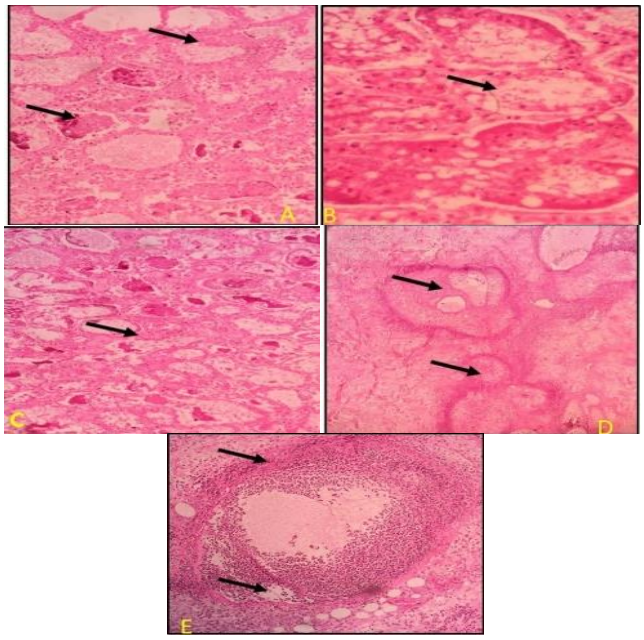

Figure 2: Micrograph of sheep mammary gland (A) showed coagulative necrosis in acini (H&E, 10 X). (B) sloughing of epithelial cells lining the alveoli with serous fluid (arrow) (H&E, 10X). (C) vacuolar degeneration of epithelial cells (H&E, 10X). (D) accumulation of proteinaceous serous material in the alveoli (H&E, 10X). (E) microabscesses (H&E, 10X).

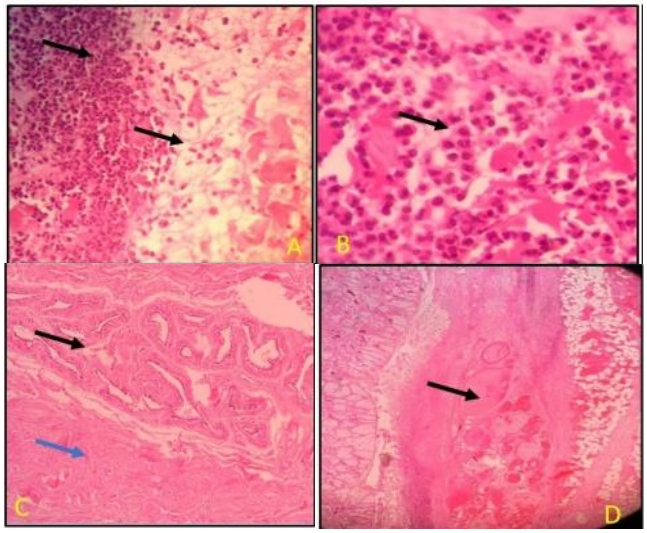

Figure 3: Micrograph of ewes mammary gland showed (A and B) chronic infiltration of an inflammatory cell (H&E, 10X). (C) infiltration of plasma cells, lymphocytes, and giant cells (H&E, 10X). (D) showed (A) atrophy (arrow)and metaplasia of alveoli epithelium (Blue-arrow) (H&E, 10X) (B) metaplasia (H&E, 10X).

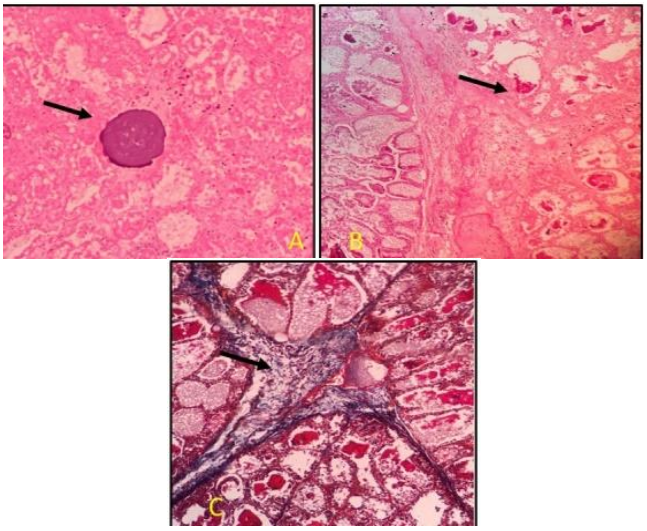

Figure 4: Micrograph of ewe's mammary gland showed (A) calcium salt deposition inside the alveoli (H&E, 10 X). (B) deposition of collagen fibrous (H&E, 10X). (C and D) thickening of interstitial tissue due to deposition of collagen fiber (Mallory's trichrome, 100x).

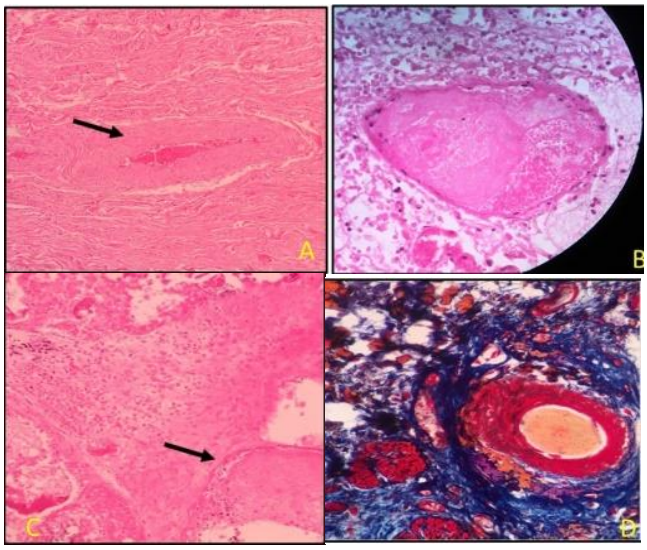

Figure 5: Micrograph of ewe's mammary gland showed (A) thickening of blood vessels wall (H&E, 40 X). (B) thrombus (H&E, 100X). (C) diffused fibrosis (H&E, 40). (D and E) Fibrosis (Masson's trichrome, 100x).

Discussion

Mastitis is the most economically significant disease of the mammary gland in ewes. These economic losses are associated with direct or indirect factors such as the cost of the treatment of the sick animals, increased mortality rates, reduced milk production, and led excessive culling of the herd (17,18). Management of the pack have been associated with multiple causes that consider as a predisposing factor for ewe's mastitis like hygiene, the conformation of the udders, healthy animal statues, suckling and prolificacy of the lamb, genetic factor (19) practices of the milk, and food, inadequate nutrition and decreased of vitamins-the causative organism like *Strep. Agalactia, S. aureus, E. coli,* and *staph spp* (20) can enter the mammary tissue in an ascending way by papillary ducts or from systemic infection (21,22).

Microscopic examination of the current study revealed acute mastitis in which the affected section showed infiltration of polymorphic nuclear leukocytes, the pathogenic bacteria reach the parenchyma of the mammary gland and access small ducts and alveoli, which then occluded by fibrin, leukocytes, and bacteria (23).

The interaction between milk leukocytes and pathogenic bacteria resulting in the establishment of inflammation (24), then macrophage release chemoattractant that attracted other polymorphonuclear cells from vasculature to the site of infection and accumulated in large numbers around the alveoli, neutrophil emigration occurs through gaps in the lining epithelia, or due to sloughing apart of it this inflammation and emigration of neutrophil are associated

with sloughing or damage of the alveoli and epithelial ducts cell (25) these result agreement with (26,27), vacuolar and coagulative necrosis can results from damage of the mammary vasculature after infection with pathogenic bacteria and these result agreement with (28,29). Chronic mastitis pathology showed severe infiltration of mono morphonuclear cells. Giant cells with collagen fiber deposition in mammary gland tissue, transforming growth factor-beta (TGF-B), play a critical role in inducing fibroblast to synthesize collagen (30). These results agree with (31) that these lesions are due to progression of infection with pathogenic bacteria, leading to an increase in the number of macrophages and stimulation of fibroblast activity that resulted in damage of alveolar epithelium decreasing in secretary activity of it. Further, multiplication of connective tissue to the large ductal lumen and glandular intestinal tissues (32), at the last stage of mastitis, there was thickening of the alveolar wall which become cornified and hypertrophied and this agreement with (33,34), who mention similar changes in the mammary gland.

Chronic mastitis can result in squamous metaplasia as a compensatory mechanism of alveolar epithelial loss. These pathological alterations agree with (35), who mentions that metaplasia leads to reducing the function of the cell through changes in the type of cell that give more protection to tissue. Calcium deposition in the mammary gland parenchyma results from Glycoprotein or cellular debris (36,37). Calcium-binding protein may become subsequently binding to such residue. Calcium-binding proteins would build up in this fashion, helping to maintain mineral deposition but not causing calcification. Mineralization is known to be triggered by bacterial infection and cellular debris (38-40).

Conclusion

We concluded from our results that ewe's mastitis could be considered an acute sheep disease that affects the function of udders and mammary glands, leading to loss of animal life. Prevention is necessary as treatment, repetitive monitoring, and periodical exam are recommended for all dairy herds to detect mastitis.

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Conflict of interest

The author advertises that there is no collision related to this research.

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دراسة التغيرات المرضية والكيميائية النسجية لالتهاب الضرع في الأغنام

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الخلاصة

يعتبر التهاب الضرع في الأغنام من المشاكل التي تسبب خسائر اقتصادية من خلال تأثيره على إنتاج الحليب فضلا على تأثيره على نوعية الحليب المنتج الذي يكون غير صالح للاستهلاك البشري. هدفت الدراسة الحالية إلى تسليط الضوء على التهاب الضرع في الأغنام المربأة في مناطق مختلفة لمدينة الموصل. أظهرت نتائج الدراسة الحالية وجود تغيرات عيانية ونسجية عديدة تمثلت التغيرات العيانية بكبر حجم الضرع وتضخمة مع وجود تغيرات في اللون والملمس فضلا عن وضوح تؤسف وانسلاخ الجلد مع وضوح النضح فيه، وبخاصة في الحالات المتقدمة بالإصابة، فيما أظهرت عينات أخرى وجود عقيدات صلبة على الضرع. اظهر الفحص النسجي وجود تغيرات نسجية مختلفة تمثلت بالتغيرات التنكسية والنخرية والتي مثلت ٣,٥% من العينات فيما كانت نسبة العينات التي تعاني من النخر الاماعي والخراجات المرتشحة بالخلايا الالتهابية هي ٢١,٤% وكانت نسبة الالتهاب العقيدي بالضرع ٢٥% فيما أظهرت عينات أخرى إصابة الضرع بالضمور والحوول وبنسبة ٢١% كما أظهرت العينات وجود خثرة دموية مع تثخن جدار الأوعية الدموية وبنسبة ١٤,٢% فيما كانت نسبة ترسب أملاح الكالسيوم١٤,٢%، نستنتج من الدراسة الحالية بان التهاب الضرع في الأغنام شائع وواسع الانتشار في الحيوانات وفي مناطق مختلفة من مدينة الموصل.