

Pathological Observations on Uterine and Mammary Gland Lesions due to *Corynebacterium* Infection in Slaughtered Ewes

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Abstract

One thousand uteri and mammary glands of sexually mature non-gravid ewes slaughtered at El-Basateen and El-Mounib abattoirs of Cairo and Giza provinces were studied. Out of these examined cases, one hundred uteri and fifty-five mammary glands showed pathological lesions *Corynebacterium pyogenes* was isolated from 21 uteri (21%) and 14 mammary glands (25.45%). Grossly, the uteri were congested and filled with greenish-white pus in their lumens. In some cases, large abscesses were also seen. Microscopically, degeneration and desquamation of the endometrial epithelium mixed with neutrophils in the uterine lumen were observed. Vasculitis with hyperplasia of the glandular epithelium, periglandular fibrosis and haemosidrosis were detected. In mammary glands, single and multiple abscesses filled with creamy to greenish-white pus were grossly seen. Microscopically, these abscesses formed from central necrotic tissue surrounded by connective tissue capsule infiltrated mainly with neutrophils. Moreover, degeneration and desquamation of the alveolar epithelium with fibrosis and mononuclear cells infiltration of the interalveolar stroma were found. The Histopathological examination of supramammary lymph nodes showed abscesses formation with dystrophic calcification. *Corynebacterium ovis* was isolated from nine uteri (9%) and three mammary glands (5.45%). Macroscopically, the uteri were enlarged, congested and showed multiple abscesses. Microscopically, congestion with degeneration and desquamation of the endometrial epithelium were observed. Hyperplasia of the glandular epithelium and periglandular fibrosis with neutrophilic infiltration were seen. Multiple abscesses with dystrophic calcification were also observed. Focal hemorrhages with hypertrophy and hyalinization of the blood vessel walls were also seen. In mammary gland, single and multiple abscesses with fistulae discharging pus were grossly noticed. Microscopically, chronic abscesses with degeneration and desquamation of acinar epithelium mixed with eosinophilic cellular debris in the acinar lumina were seen. Moreover, no pathological changes were detected in the supramammary glands of these udders

Introduction

Sheep production and reproduction depend upon the health and fertility of reproductive organs specially those of female. The commonest recorded pathological uterine lesion in ewes was endometritis (6 and 17). *Corynebacterium pyogenes* was one of the most commonly isolated microorganisms in case of endometritis (6 and 11), while, *Corynebacterium ovis* was recorded in metritis, uterine edema and caseous lymphadenitis of the genital lymph nodes of ewes (21). Mastitis is a major problem in ovine mammary glands causing great economic losses due to reduction in milk yield and lowering of its nutritive value (18). Mastitis usually manifested grossly by swelling of the udder and in the later stage the udder became necrotic and may slough off (16). Mastitis caused by various bacterial agents mainly *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Corynebacterium pyogenes*, *Pasteurella haemolytica*, *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* (18 and 9). Acute and chronic inflammation of udder involving tissue supramammary lymph nodes were recorded in *Corynebacterium pseudotuberculosis* infection in ewes (25 and 3).

The present study was carried out in order to record the incidence of Corynebacterial infections among slaughtered ewes and to describe both gross and histopathological changes in the uterus and mammary gland met with this infection.

Material and Methods

Uteri, mammary glands and the related draining supramammary lymph nodes from were collected from 1000 sexually mature non gravid ewes slaughtered in El-Basateen and El-Mounib abattoirs of Cairo and Giza provinces with history of infertility during the period from January 2004 to December 2005.

For histopathological examination, tissue specimens from these organs were rapidly fixed in 10% neutral buffered formalin solution. After

proper fixation, tissue-paraffin sections about 4-6 um were routinely prepared and then stained with HandE stain for microscopic examination (12). Moreover, special staining techniques such as Crossman's trichrome stain, Von-Kossa's stain and Prussian blue stain were also performed (7).

For bacteriological examination, samples from uteri, mammary glands and supra-mammary lymph nodes of the examined ewes were taken individually in plastic bags and sent to laboratory under refrigeration for isolation and identification of the causative bacterial agents. Identification of the isolates was depending on the results of morphological findings, culture characters of the colonies as well as biochemical tests (8).

Results

Incidence of *Corynebacterium* infection among uterus and mammary glands of slaughtered ewes:-

A total number of 1000 uteri and 1000 mammary glands of ewes slaughtered in El-Basateen and El-Mounib abattoirs during the period from January 2004 to December 2005 were grossly examined. From these examined uteri and mammary glands, 100 uteri and 55 mammary glands showed different gross lesions. Out of these 100 uteri, *Corynebacterium pyogenes* were isolated from 21 uteri with an incidence of 21% and *Corynebacterium ovis* were isolated from 9 cases with an incidence of 9%. Meanwhile, Out of these 55 mammary glands, *Corynebacterium pyogenes* were isolated from 14 cases with an incidence of 25.45% and *Corynebacterium ovis* were isolated from 3 mammary glands with an incidence of 5.45% (Table 1).

Table (1): Number and percentages of *Corynebacterium pyogenes* and *Corynebacterium ovis* isolated from uteri and mammary glands of ewes slaughtered at Cairo and Giza abattoirs.

Etiology	uterus		Mammary gland	
	number	%	number	%
1. <i>Corynebacterium pyogenes</i>	21	21	14	25.45
2. <i>Corynebacterium ovis</i>	9	9	3	5.45
Total	30	30	17	30.90

1. *Corynebacterium pyogenes* infection:-

A-Uterus:

Macroscopically, the uteri of most of the affected cases were enlarged, edematous, congested, doughy in consistency and showed grayish-white patches on their surfaces. Cut section revealed creamy grayish-white to greenish-white pus flow out on the uterine mucosa (Fig.1). The endometrium of some examined uteri showed multiple small cysts contained clear watery fluid. Moreover, in few examined uteri large sized abscesses of about 5 cm in diameter were seen on the uterine body and oozing creamy greenish-white pus on their cut-sections.

The histopathological examination of the uteri revealed desquamation of the endometrial lining epithelium with edema and leucocytic cellular infiltration of endometrium particularly lymphocytes. Cystic dilatation of some endometrial glands with accumulation of eosinophilic cellular debris in their lumina was seen (Fig.23). Degenerative changes of the lining epithelium of the endometrial glands with periglandular fibrosis which gave positive reaction by Crossman's trichrome stain were observed (Fig.3, A and B). Hyalinized desquamated epithelial cells intermixed with inflammatory cells particularly neutrophils were seen in the lumens of some endometrial glands. Aggregations of amorphous brownish granules of hemosiderin pigments that reacted positively with Prussian blue stain were observed in-between the endometrial glands (Fig. 4, A and B). Severe congestion of the myometrial blood vessels and

hyalinization of their muscular layers with damage and/or hyperplasia of the endothelium were recorded. Moreover, mononuclear inflammatory cellular infiltration of the endometrium and in-between the myometrial bundles was seen. Moreover, multiple abscesses formed from central necrotic area surrounded with inflammatory cells mainly neutrophils and mononuclear cells were also seen in endometrium of some examined uteri (Fig. 5).

Mammary gland:

Macroscopically, most of the affected mammary glands showed single or multiple abscesses about 1-2 cm in diameter embedded in their parenchyma (Fig. 6). The cut surfaces of these abscesses revealed sticky, creamy grayish-white to greenish-white pus. Moreover, pus of offensive odor was recorded in some mammary glands.

Microscopically, the picture of focal suppurative mastitis was prevalent and represented by the presence of large sized chronic abscesses formed from finely granular eosinophilic and basophilic structureless necrotic centers surrounded with inflammatory zones mostly neutrophils, lymphocytes and macrophages, and enclosed by fibrous connective tissue capsule infiltrated with neutrophils and lymphocytes (Fig. 7). Some of these abscesses showed dystrophic calcification appeared as purplish irregular masses in the center of these abscesses. Severe destruction and necrosis of the remaining alveolar tissues with intra-alveolar neutrophilic cellular infiltration intermixed with desquamated epithelial cells were found (Fig. 8). In addition, some alveolar luminae were filled with eosinophilic exudate and other contained irregular bluish masses of calcium salts. Severe fibrous connective tissue proliferation and leucocytic cellular infiltration of the interalveolar stroma mainly mononuclear type were also observed (Fig. 9). Moreover, the examined lactiferous ducts showed desquamation of the lining epithelium with leucocytic cellular aggregation in their lumens.

Supramammary lymph nodes:

Grossly, the supramammary lymph nodes of the affected mammary glands were enlarged and firm. Large sized abscesses up to 1-2 cm in diameter containing grayish-white pus were seen in lymph nodes of two affected mammary glands (Fig.10).

Histopathologically, severe edematous thickening of the capsules and trabeculae of the lymph nodes was found. Multiple abscesses formed from central necrotic areas surrounded by mononuclear cells and fibrous connective tissue capsules were detected. Some of these abscesses showed central dystrophic calcification taken blackish coloration with Von-Kossa's stain (Fig.11 A and B).

2. *Corynebacterium ovis* infection:-

A-Uterus:

Grossly, the affected uteri were enlarged, doughy in consistency. The serosa was congested and in some cases petechial hemorrhages were seen. The mucosa showed grayish-white foci, which gave gritty feeling on cut sections. Many endometrial cysts were seen in some uteri. In some cases, opening of the uterus revealed grayish-white pus flow out on the affected uterine mucosa. This pus varied in amount and consistency but it was mostly creamy. Moreover, sticky pus with cheesy materials adhered to the uterine mucosa was seen in two examined uteri (Fig.12).

Microscopically, desquamation of the lining epithelium of the endometrium with accumulation of desquamated epithelial cells intermixed with neutrophils in the uterine lumen were predominant. In addition, hydropic degeneration of the lining epithelium of endometrium was recorded in two examined uteri. The examined endometrium showed congestion, edema, haemosidrosis and leucocytic cellular infiltration mainly neutrophils and macrophages which extended to myometrium. Focal area of dystrophic calcification surrounded with mononuclear leucocytic cellular infiltration was also detected (Fig.13). Cystic dilatations of the endometrial glands with hyperplasia of their lining epithelium and periglandular fibrosis were seen (Fig.14). Severe endometrial hemorrhages with hypertrophy and hyaline degeneration of the muscular layers of some uterine blood vessel were detected (Fig.15). Moreover, small mononuclear cells aggregation was also found.



Fig 1

Fig 2

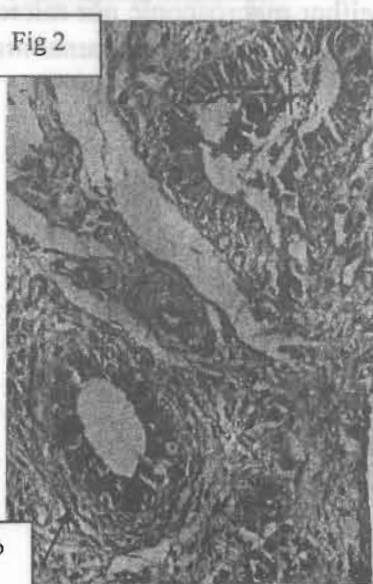


Fig 3a

Fig 3b

Mammary gland:

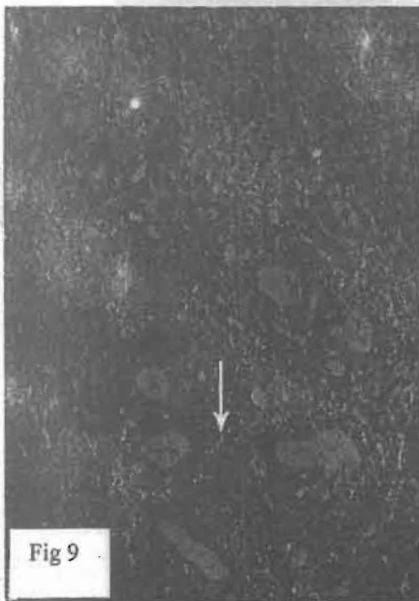
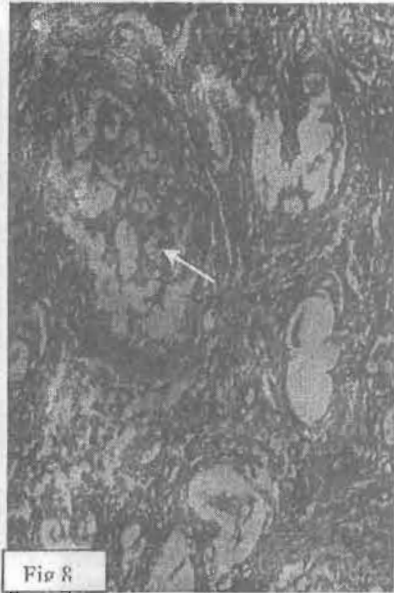
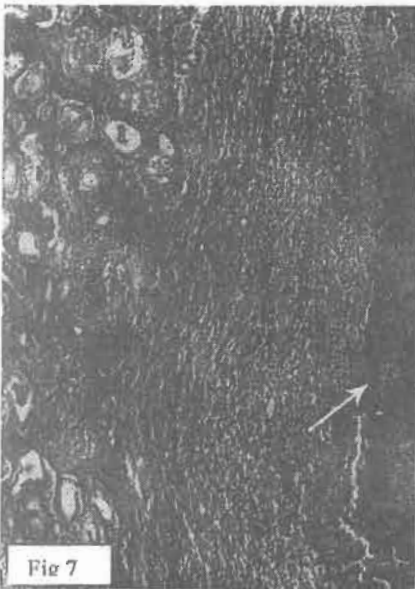
The gross examination of mammary glands revealed single or multiple chronic abscesses embedded in their parenchyma. Most of these abscesses showed central dry cheesy materials (Fig.16). Cut sections of some of these abscesses gave gritty sound. Fistula formation discharging pus was observed in some of these abscesses.

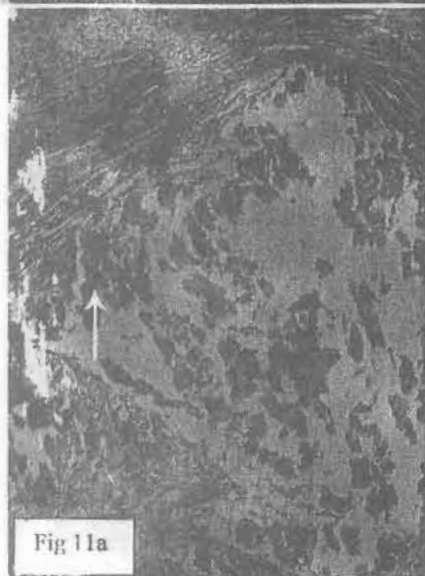
Microscopically, abscesses formed from central finely granular eosinophilic and basophilic structureless necrotic debris and dystrophic calcification surrounded with heavily mononuclear inflammatory cells and fibrous connective tissues were observed. The alveoli in the vicinity of these abscesses showed eosinophilic exudate intermixed with desquamated epithelium in their lumens (Fig.17). Moreover, focal mononuclear inflammatory cellular infiltration of the alveoli was also detected.

Supramammary lymph nodes:

Neither macroscopic nor microscopic pathological changes were recorded in the examined supramammary lymph nodes in cases of *Corynebacterium ovis* infection.









Discussion

In the present study, out of 1000 examined uteri and mammary glands of ewes slaughtered at El-Basateen and El-Mounib abattoirs, 100 uteri and 55 mammary glands showed different pathological lesions. In this work, *Corynebacterium pyogenes* was isolated from 21 uteri (21%). These result was partial in agreement with those recorded by (20) 42%; (11) 12.66% and (5) 18.4%. Concerning to the associated uterine lesions with *Corynebacterium pyogenes* infection in the present work, the affected uteri were grossly enlarged, congested and showed greenish-white pus in the uterine lumens. These changes were recorded previously by (23) in goat and (6) and (11) in sheep and in the same time were partially agree with (14) who reported reddish-brown pus in cattle suffered from *Corynebacterium pyogenes* infection. Moreover, in the present work, large abscesses were seen on the uterine body walls in some cases of *Corynebacterium pyogenes* infection. This gross picture was completely agreed with (24); (16) and (15). In this investigation, microscopic examination of the uterus revealed degeneration and desquamation of the lining epithelium of the endometrium and endometrial glands with neutrophilic cellular infiltration of the stroma. Hyperplasia of the endometrial glands and periglandular fibrosis were also seen. These results were in a harmony with that reported by (24), (6) and (11). In our results, old abscesses formed from central necrosis surrounded by inflammatory cellular reaction and connective tissue capsule were noticed. These microscopic findings were also mentioned by (24) and (16). However, these results were partial in agreement with (4) who found chronic endometritis and (14) who reported endometritis, metritis and pyometra in *Corynebacterium pyogenes* infection.

Moreover, vascular changes represented by congestion, injury of the endothelium and hyalinization of the tunica muscularis of the uterine blood vessels were also observed in the uterus in *Corynebacterium pyogenes* infection. This result was in completely agreed with those of (11) in ewe, and partially agreed with (14) who reported congestion and thrombosis without degenerative changes in blood vessels of uterus of cow in *Corynebacterium pyogenes* infection.

In the present study, *Corynebacterium pyogenes* was isolated from 14 ovine mammary glands with an incidence of 25.45%. This incidence was nearly the same to that prior reported by (9) 23.33% and in the same time it was higher than that of (1) 4%. Concerning to the gross findings of these examined mammary glands, single and multiple abscesses filled with greenish-white pus were observed. These findings were completely in agreement with that recorded (16). On contrary our results were partially agreed with (9) who reported enlarged turgid mammary glands with smooth pale cut-section. In the present work, the histopathological examination of these mammary glands in *Corynebacterium pyogenes* infection revealed abscesses formed from center necrotic areas surrounded by fibrous connective tissue capsules infiltrated with neutrophils and mononuclear cells. Our results were in a partial agreement with (22) who recorded small sized organized abscesses in the interlobular septa. In the present investigation, microscopic changes including degeneration and desquamation of the alveolar epithelium with fibrosis and mononuclear leucocytic cellular infiltration of the inter-alveolar stroma were also seen. Similar microscopic picture was also reported by (16), and (9). Meanwhile, our result was nearly similar to that reported by (13) who found the same lesions in addition to hyperplasia and squamous metaplasia of the interlobular collecting duct epithelium.

In the present work, the supramammary lymph nodes in cases of *Corynebacterium pyogenes* infection were enlarged and showed abscesses formation oozing grayish-white pus on sectioning. These macroscopic findings were in partial agreement with (13) who found enlargement, congestion and edema of lymph nodes and (9) who reported only enlarged

lymph nodes. Microscopic examination of lymph nodes in cases of *Corynebacterium pyogenes* infection revealed suppurative lymphadenitis characterized by abscess formation with dystrophic calcification. These findings were partially in accordance with (9) who reported mononuclear cellular infiltration with hyperplasia of the lymphoid follicles and medullary cords.

In this study, *Corynebacterium ovis* was isolated from 9 uteri with uterine lesions with an incidence of 9%. Unfortunately, literatures concerning to *Corynebacterium ovis* infection of uterus were very scant. However, *Corynebacterium ovis* was isolated previously from uterine lesions of ewe (21 and 25). In this work; the affected uteri were grossly enlarged, doughy, congested and filled with pus. This result was completely agreed with that mentioned by (25) and partial in agreement with (21) who recorded edema and serosanguinous exudates in the uteri. Microscopically, in the present work, the endometrium showed congestion, hemorrhages, degeneration and desquamation of the endometrial epithelium with hyperplasia of the endometrial glands. Periglandular fibrosis and neutrophilic infiltration were also seen. Moreover, multiple abscesses formed from necrotic tissue and dystrophic calcification surrounded by inflammatory zone was observed. These microscopic observations were in a partial agreement with (16) who reported the same lesions without calcification.

In the present work, *Corynebacterium ovis* was isolated from three ovine mammary glands with an incidence of 5.45%. This result was nearly the same as mentioned by (2) in goat and lower than that reported by (9) 10% in ewe. In the present work, gross examination of the affected mammary glands showed single and multiple abscesses with fistulae discharging cheesy pus. These findings were in a harmony with that aforementioned by (25), (3) and (9). Microscopically, the examined mammary glands revealed chronic abscesses formed from central necrosis and calcification surrounded by inflammatory reaction and fibrous capsule. The remaining alveoli showed desquamation of their epithelium and accumulation of eosinophilic cellular debris in the alveolar lumina. These

results were in a partial agreement with that of (13), (16), (3) and (9) who recorded the same lesions without calcification.

Finely the present investigation concluded that, *Corynebacterium* infection of ewe was accompanied with suppurative inflammation of uterus and mammary glands with abscesses formation mostly in mammary glands and involved the drained supramammary lymph nodes in *Coynebacterium pyogenes* infection.

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مشاهدات باثولوجية على التغيرات المرضية بالرحم والضرع نتيجة الإصابة
بميكروب الكوريني بكتريم فى النعاج المنبوحة

عبد الرحيم امين ناجى - محمود سالم جاب الله - عبد الباسط اسماعيل المشد - أحمد عبد الحافظ
طنطاوى - محمد جودة برعى
قسم الباثولوجيا - كلية الطب البيطرى - جامعة بنها

الملخص العربى

تتلو هذه الدراسة فحص ارحام وضروع عدد الف نعجة نُبحت بمجزرى البساتين والمنيب بالقاهرة
والجزيرة فى الفترة من يناير ٢٠٠٤ الى ديسمبر ٢٠٠٥ وقد سجلت مائة اصابة مرضية بالارحام وخمسة
وخمسين بالضرع، تم عزل ميكروب الكوريني بيوجينز من ٢١ رحم مصاب (٢١%) ، ١٤ ضرع مصاب
(٢٥.٤٥%)، وقد أظهر الفحص العيى للرحم فى هذه الحالات وجود احتقان وجمع صديدي فى تجويفه
مع خرايج فى بعض الحالات. وبالفحص المجهرى شوهد تساقط الخلايا الطلائية المبطنه لجدار الرحم
والتي تخللتها خلايا التهابية فى تجويف الرحم. كذلك لوحظ فرط نسيجى فى الطبقة الطلائية المبطنه للغدد
الرحمية بالاضافة الى تراكم من صبغة الهيموسيدرين وزيادة كمية النسيج اللينى حول الغدد الرحمية، كما
شوهد أيضا خرايج مزمنة مع التهاب بالاووعية الدموية. وبالنسبة للضرع المصابة، فقد أظهرت الدراسة
العينية وجود خراج أو عدة خرايج مملوءة بالصديد. وبالفحص المجهرى وجد أن هذه الخرايج تتكون من
مناطق تنكز محاط بنسيج لينى يتخلله خلايا التهابية متعددة النواة. كما لوحظ وجود تغيرات فسادية
وتساقط فى الخلايا الطلائية للحويصلات الغدية مع زيادة فى النسيج اللينى بين الحويصلات الغدية يتخلله
كثير من الخلايا الالتهابية أحادية النواة. أما عن العقد الليمفاوية الفوق ضرعية فقد وجد بها خرايج فى
النسيج الليمفاوى تتخللها ترسبات كالمسبة. كما تم عزل ميكروب الكوريني أوفز من تسعة أرحام مصابة
(٩%) و ثلاثة ضروع مصابة (٥.٤٥%). وقد أظهر الفحص العيى للرحم المصاب زيادة فى حجمه،
واحتقانه ، ووجود خرايج به تحتوى على صديد. وبالفحص المجهرى، تبين وجود احتقان مع تغيرات
فسادية وتساقط فى الخلايا الطلائية للطبقة المبطنه للرحم و فرط التنسج للغدد الرحمية، كما وجد زيادة فى
كمية النسيج الالينى حول الغدد الرحمية تتخلله أعداد كبيرة من الخلايا الالتهابية متعددة النواة. كما لوحظ
وجود ترسبات كالمسبة وخرايج. بالاضافة الى ذلك فقد شوهد مناطق أنزفة مع تضخم وترسبات زجاجية
فى جدران الأوعية الدموية. وقد أوضح الفحص العيى للضرع المعزول منها هذا الميكروب، ووجود
خرايج كبيرة وصغيرة تحتوى على صديد متجبين. وقد أظهر الفحص المجهرى وجود خرايج مزمنة
بالاضافة الى تغيرات فسادية وتساقط فى خلاياها الطلائية للحويصلات الغدية وامتلائها برواسب وردية. ولم
تسجل اى تغيرات مرضية سواء عينية أو مجهرية فى العقد الليمفاوية الفوق ضرعية فى هذه الحالات.