Mastitis pathogens in relation to histopathological changes in buffalo udder tissues and supramammary lymph nodes

By

Abeer, E. El-Metwally* and Hanaa, A.E. Asfour **

*Pathology Department, Animal Reproduction Research Institute (A.R.R.I.)
**Mastitis & Newnate Department, Animal Reproduction Research Institute (A.R.R.I.)

SUMMARY

A total of 112 samples of mammary glands and supramammary lymph nodes were examined for microbiological and histopathological examination. Isolated microorganisms were *S. aureus*, *CNS*, *Streptococcus* spp., *coli form* spp. Histopathological examination revealed acute and chronic mastitis. Lymph nodes showed marked depletion, suppurative lymphadenitis with oedema and haemorrhage. Isolated fungi were *Cladosporium* spp., *Penicillium* spp., *Phialophora* spp., *Aspergillus* spp., *Paecillomyces*, *Helminthosporium*, *Althrosperes*, *Epicoccum* and *Stemphillium*. Microscopically, acute mycotic cases showed infiltration of inflammatory cells with destruction of acinar epithelium while chronic mycotic changes in mammary tissue were characterized by focal or diffuse granulomatous type. Madin Darby Bovine Kidney cell line (MDBK) were used in this work, to examine the adherence and effect of selected bacteria within 24 h at 37°C incubation. The cytopathic effect of *S. aureus* on cell line was stronger than that of *E. coli* and *S. agalactiae*.

INTRODUCTION

Mastitis is an inflammation of the mammary gland involving either the secretory cells or the connective tissue or both. It is characterized by physical, chemical and usually microbiological changes in the milk and pathological changes in the glandular tissue (Refai, 1988). The economic significance of the disease varies among herds and to some extent depends on the system of management and degree of intensification. In addition, mastitis may have
some public health importance, since occasionally milk harbouring human pathogens may cause infection to the consumers of raw or inadequately heated milk. Mastitis remains unsolved problem because of its complex aetiology (Harby et al., 1991). Decreased milk production accounts for approximately 70% of the total cost of mastitis (Zhao and Lacasse, 2008).

Bacteria are believed to be the major cause of mastitis in cattle, buffaloes, goats, etc., but presently fungi are also recognized as primarily aetiological agents of mastitis. The incidence of mycotic mastitis appears to be increased, because of contamination of animal ration as well as the extensive indiscriminate use of antibiotics (El-Rashidy et al., 1996).

Lymph nodes are considered to be defensive barriers against pathogenic microorganisms, which brings about degenerative, inflammatory and/or hyperplastic changes (El-Mahdy et al., 2002).

So, the present study was planned to throw a light on some pathological affections of mammary glands and their lymph nodes in slaughtered buffaloes in Egypt and to identify its related causative pathogens.

**MATERIAL AND METHODS**

**A- Sample:** A total of 112 mammary tissues and supramammary lymph nodes were collected from Giza abattoirs during 8 months (February to December 2007) from slaughtered buffaloes; their ages were 5-10 years old. Each sample was divided into two parts, one part was put in a small polyethylene bag in an ice box under aseptic conditions for bacteriological examination and the second part was immersed in 10% neutral buffered formalin solution for histopathological evaluation.

**B- Histopathological examination:** Specimens from mammary parenchyma and lymph nodes were routinely processed, embedded in paraffin wax, sectioned at 4μ and stained with Hematoxylin & Eosin. Prussian blue stain, Gomori Methenamine Silver stain (GMS) and Periodic Acid Schiff stain (PAS) were used as special stains (Bancroft and Marilyn, 2002).

**C- Microbiological examination:**

1- **Bacteriological examination:** The collected specimens were cultured into nutrient broth for 24 h at 37°C. A loopfull was inoculated onto the following media, 5% defibrinated sheep blood agar, MacConkey and nutrient agar. The suspected colonies were picked up and subcultured onto the selective media for identification by microscopic examination and biochemi-
cal reactions using standard bacteriological methods according to Quinn et al. (2002).

**2-Mycotic examination:**- Inoculated plates of sabouraud dextrose agar containing antibiotic were incubated at 25°C for 7 days. The first examinations of the plate were done after 2 days to determine the degree of fungal growth. Representative growth isolated on sabouraud dextrose agar slopes for further identification according to Samson (1979).

D-The effect of *S. aureus*, *E. coli* and *S. agalactiae* on monolayer cell line culture: The main causes of mastitis of the examined cases in this work were *S. aureus*, *S. agalactiae* and *E. coli*, which were used for detection of their cytopathic effect on Madin Darby Bovine Kidney cell line (MDBK). Cell line were kindely supplied by the department of Virology “Animal Reproduction Research Institute”.

**Cell line preparation:** This test was carried according to Saad et al. (2000). MDBK Cells were grown in tissue culture test tubes and maintained on MEM growth medium supplemented with 10% fetal calf serum "FCS" at 37°C in CO2 incubator. Bacterial suspension (10^5-10^6 cfu/ml) was added to each tube then incubated at 37°C for 24 hours and the monolayers were washed 5 times by PBS. Effect of bacteria was detected in different times. The tubes were washed, fixed in acetone solution, stained with Gram stain (crystal violet for G +ve bacteria & carbon fuchsin for G -ve bacteria) and examined by inverted light microscopy.

**RESULTS**

Bacteriological investigation revealed that the isolated microorganisms from the mammary glands and related lymph nodes were *S. aureus* (47 cases), *Coagulase Negative Staphylococci* (CNS) (47 cases), other streptococci (other than *S. agalactiae*) (22 cases), *Proteus vulgaris* (19 cases), *S. agalactiae* (13 cases), *E. coli* (8 cases) and *Citrobacter freundii* (6 cases), as single and/or mixed infection.
Table (1): Showing types of bacterial isolates from mammary tissues and regional lymph nodes of buffaloes.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of samples</th>
<th>% from total (112)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A-Single infection:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1- <em>S. aureus</em></td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>2- CNS</td>
<td>24</td>
<td>21.4</td>
</tr>
<tr>
<td>3- <em>E. coli</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>B-Mixed infection:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1- <em>S. aureus</em> + other <em>Streptococci</em></td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>2- <em>S. aureus</em> + <em>Proteus vulgaris</em></td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>3- <em>S. aureus</em> + <em>S. agalactiae</em></td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>4- <em>S. aureus</em> + <em>Citrobacter freundii</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>5- CNS + other <em>Streptococci</em></td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>6- CNS + <em>Proteus vulgaris</em></td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>7- CNS + <em>E. coli</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>8- CNS + <em>S. agalactiae</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>9- <em>E. coli</em> + <em>S. agalactiae</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>10- <em>Proteus vulgaris</em> + other <em>Streptococci</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>11- <em>S. aureus</em> + <em>Citrobacter freundii</em> + <em>S. agalactiae</em></td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>12- <em>S. aureus</em> + <em>E. coli</em> + other <em>Streptococci</em></td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>13- <em>S. aureus</em> + <em>E. coli</em> + <em>S. agalactiae</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>14- <em>S. aureus</em> + <em>Citrobacter freundii</em> + other <em>Streptococci</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>15- <em>S. aureus</em> + <em>Proteus vulgaris</em> + <em>S. agalactiae</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>16- <em>S. aureus</em> + <em>Proteus vulgaris</em> + other <em>Streptococci</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>17- CNS + <em>Proteus vulgaris</em> + other <em>Streptococci</em></td>
<td>6</td>
<td>5.3</td>
</tr>
<tr>
<td>18- CNS + <em>Proteus vulgaris</em> + <em>S. agalactiae</em></td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>19- CNS + <em>E. coli</em> + <em>S. agalactiae</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>20- CNS + <em>E. coli</em> + other <em>Streptococci</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>21- CNS + <em>Citrobacter freundii</em> + <em>S. agalactiae</em></td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Bacteriologically negative</strong></td>
<td>15</td>
<td>13.3</td>
</tr>
</tbody>
</table>
1- *Staphylococcus aureus* mastitis:

Macroscopically, most of the mammary glands were lactating, showing congestion with haemorrhage and oozing turbid purulent secretion while few cases were fibrosed with pale discolouration. Most of lymph nodes appeared normal and some cases were oedematous while others showed congestion with minute petechial haemorrhages detected on its cut surface.

Microscopically, acute mastitic cases showed desquamation of epithelium into the lumen of acini with vacuolar degeneration. Fibrin network and caseated milk in acini were observed. The mammary acini and interstitial connective tissue were highly infiltrated with inflammatory cells mainly neutrophils, macrophages, histiocytes and plasma cells. Vasculitis with edema were detected. In few cases, there were focal haemorrhages into some alveoli and interstitial connective tissue (**Figs. 1 & 2**). Lymph nodes of these cases showed marked depletion with destruction of center of lymphoid follicles. Suppurative lymphadenitis with vasculitis and subcapsular inflammatory edema were observed. Hemorrhage with haemosidrin pigments were detected which were confirmed with Prussian blue stain (**Figs. 3 & 4**).

Chronic mastitis was observed in few cases which were characterized by proliferation of fibrous connective tissue and infiltrated with lymphocytes, macrophages and plasma cells. Vacuolar degeneration of acinar epithelium with cystic dilatation of acini was observed. Corpora amylacea were detected inside acini and some of them were calcified. Hyperplasia of epithelial covering of ductular epithelium was detected. Blood vessels showed thickening of tunica media with aggregation of inflammatory cells around them. Marked depletion was observed in related lymph nodes.

2- *Coagulase Negative Staphylococcus* mastitis:

Macroscopically, most of mammary glands were apparently normal while few cases were fibrosed and calcified. Most of examined lymph nodes appeared normal except few cases showed severe congestion with yellowish grey caseated content.

Microscopic appearance of most of glands showed vacuolar degeneration of mammary epithelium which is the most prominent sign. Few cases showed acute mastitis with hemorrhage and congestion while other few cases showed chronic mastitis (**Fig. 5**). Lymph nodes revealed marked depletion with destruction of center of lymphoid follicles and subcap-
sular edema. Acute suppurative lymphadenitis with haemorrhage and haemosidrosis were observed. Meanwhile inspissated pus was seen in some other foci.

3- **Streptococcus mastitis:**

Macrosopically, some cases were congested with dark colouration and others were pale and fibrosed. No characteristic lesions were detected in related lymph nodes except slight congestion in some cases.

Microscopic appearance of *S. agalactiae* mastitis showed acute suppurative mastitis with presence of corpora amylacea (Figs. 6 & 7). Inflammatory cells aggregation was detected at subepithelial layer of lactiferous ducts. Related lymph nodes showed marked depletion, acute suppurative lymphadenitis, inflammatory oedema, severe congestion and haemorrhage with haemosidrosis (Fig. 8).

Streptococci other than *S. agalactiae* mastitis were characterized by vacular degeneration and desquamation of epithelium with inflammatory cells aggregation. Few cases showed subacute mastitis with mild fibrosis while some lobules showed marked focal chronic mastitis. Lymph nodes of these cases showed marked depletion with vasculitis while some of them showed activation of lymphoid follicles with hypertrophy of wall of blood vessels.

4- **Coliform mastitis:**

No characteristic lesions were detected macroscopically except congestion and haemorrhage.

Microscopic appearance of *E. coli* mastitic cases showed severe, acute, suppurative necrotizing mastitis in which strands of fibrin with cellular debris and caseated milk inside alveoli were evident. There were interstitial edema, hemorrhage and moderate to severe congestion (Fig. 9). Few cases showed chronic mastitis with cystic dilatation of alveoli.

In case of other coliform (*Proteus vulgaris & Citrobacter freundii*), there were mild congestion and aggregation of inflammatory cells. Few cases showed acute mastitis while others showed chronic mastitis.

Acute suppurative lymphadenitis with haemorrhage and haemosidrosis were observed in all of examined lymph nodes (Fig. 10).

II- **Mycotic mastitis:**

Isolated fungi were *Cladosporium spp.* (15 cases), *Penicillium spp.* (14 cases), *Phialophora spp.* (11 cases), *Aspergillus fumigatus* (11 cases), *Aspergillus flavus* (5 cases), *Aspergillus candidiasis* (4 cases), *Paecilomyces* (4 cases), *Helminthosporium* (4 cases), *Althrospos* (2 cases), *Epicoccum* (1 case) and *Stemphillium* (1 case) as single and/or mixed infection (Fig. 11).
Table (2): Showing types of isolated mould from mammary tissues and regional lymph nodes of buffaloes.

<table>
<thead>
<tr>
<th>Mould species</th>
<th>No. of samples</th>
<th>% from total (112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Single infection:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1- <em>Penicillium</em> spp.</td>
<td>14</td>
<td>12.5</td>
</tr>
<tr>
<td>2- <em>Cladosporium</em> spp.</td>
<td>4</td>
<td>3.57</td>
</tr>
<tr>
<td>3- <em>Pialophora</em> spp.</td>
<td>3</td>
<td>2.68</td>
</tr>
<tr>
<td>4- <em>Aspergillus fumigatus</em></td>
<td>3</td>
<td>2.68</td>
</tr>
<tr>
<td>5- <em>Aspergillus candidiasis</em></td>
<td>2</td>
<td>1.79</td>
</tr>
<tr>
<td>6- <em>Althrosepore</em> s</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>7- <em>Paecilomyces</em></td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>B-Mixed infection:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1- <em>Helminthosporium</em> + <em>Cladosporium</em> spp.</td>
<td>2</td>
<td>1.79</td>
</tr>
<tr>
<td>2- <em>Helminthosporium</em> + <em>Pialophora</em> spp.</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>3- <em>Helminthosporium</em> + <em>Stemphillium</em></td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>4- <em>Cladosporium</em> spp. + <em>Aspergillus flavus</em></td>
<td>3</td>
<td>2.68</td>
</tr>
<tr>
<td>5- <em>Cladosporium</em> spp. + <em>Aspergillus fumigatus</em></td>
<td>2</td>
<td>1.79</td>
</tr>
<tr>
<td>6- <em>Cladosporium</em> spp. + <em>Aspergillus candidiasis</em></td>
<td>2</td>
<td>1.79</td>
</tr>
<tr>
<td>7- <em>Cladosporium</em> spp. + <em>Althrosepore</em></td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>8- <em>Cladosporium</em> spp. + <em>Epicoccum</em></td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>9- <em>Pialophora</em> spp. + <em>Aspergillus fumigatus</em></td>
<td>4</td>
<td>3.57</td>
</tr>
<tr>
<td>10- <em>Pialophora</em> spp. + <em>Aspergillus flavus</em></td>
<td>2</td>
<td>1.79</td>
</tr>
<tr>
<td>11- <em>Pialophora</em> spp. + <em>Paecilomyces</em></td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>12- <em>Aspergillus fumigatus</em> + <em>Paecilomyces</em></td>
<td>2</td>
<td>1.79</td>
</tr>
<tr>
<td><strong>Negative cases</strong></td>
<td>62</td>
<td>55.35</td>
</tr>
</tbody>
</table>

Macroscopic appearance of most of the mammary glands was apparently normal while some cases were fibrosed and calcified with gritty sound on cut section.

All lymph nodes were apparently normal except some congestion varied from mild to severe.

Microscopically, acute my-
cotic cases showed infiltration of inflammatory cells with prominent vascular degeneration and destruction of acinar epithelium. There were fibrin network in acini and haemorrhage was seen in some acini. Diffuse necrosis observed in tissues in most of cases. Chronic foccal or diffuse granulomatous lesions were characterized by aggregation of macrophages, lymphocytes, plasma cells and eosinophils together with giant cells. Prominent corpora amylacea was an outstanding finding. Fungal hyphae and spores were detected within the necrotic tissue which confirmed by GMS &PAS stains. Lactiferous ducts showed hyperplastic proliferation of its lining epithelium with infiltration of lymphocytes and macrophages at the subepithelial layer.

Regional lymph nodes showed marked depletion with severe central destruction of their follicles. Acute suppurative lymphadenitis with haemorrhage and haemosidrosis were observed (Fig. 12). While other cases showed activation of lymphoid follicles with hypertrophy of wall of blood vessels.

III- The effect of isolated bacteria on monolayer cell line culture:

As *S. aureus*, *S. agalactiae* and *E. coli* were the main causes of mastitis in buffaloes in the present work, detection of their effect on MDBK within 24 h at 37 °C was performed (Fig. 13).

*S. aureus* was initially adhered to MDBK cells after 2 h incubation and produce large areas of erosions. After 4 h, there were adherent microorganisms to MDBK cells, forming small colonies with marked erosions. During the period between 6-24 h incubation, attachment of *S. aureus* to both damaged & living cells was observed and resulting erosions became more prominent (Fig. 14).

*E. coli* showed great cytopathic effect on MDBK which was ranged from erosion to complete cell damage. After the first 2 h, the effect of *E. coli* was mild erosion, small sized devoid areas and loss of attachment between cells. After 4 h, the size of devoid areas became more prominent. By time, the cytotoxic effect of *E. coli* became more severe as evidenced by extensive areas of erosion (Fig. 15).

The cytopathic effect of *S. agalactiae* on cell lines was milder than that of other bacteria as evidenced by mild cell damage and erosions. After 4 h incubation, there were minimal tissue damages. The latter was increased up to 24 h to a limited extent and the microorganism attached to the living cells only (Fig. 16).
Fig (1) Mammary gland affected by acute *S. aureus* mastitis showing infiltration of interstitial connective tissue with inflammatory cells mainly neutrophils, macrophages, histiocytes and plasma cells and hyperemia of interstitial blood vessels (Stain H&E, X40).

Fig (2) Mammary gland affected by acute *S. aureus* mastitis showing destruction of epithelial covering the mammary acini, fibrin network & caseated milk in acini and blood vessels dilated & engorged with blood (Stain H&E, X10).

Fig (3) Supramammary lymph node affected by acute *S. aureus* infection showing suppurative lymphadenitis, hemorrhagic exudates and interstitial edema (Stain H&E, X10).

Fig (4) High power of the previous picture showing suppurative lymphadenitis and hemorrhagic exudates (Stain H&E, X40).
Fig (5) Mammary gland affected by acute CNS mastitis showing mammary acini and interstitial connective tissue infiltrated with inflammatory cells mainly neutrophils and macrophages with destruction of the lining epithelium (Stain H&E, X40).

Fig (6) Mammary gland affected by acute *S. agalactiae* mastitis showing acute suppurative mastitis with presence of corpora amylacea and inflammatory cells aggregation inside alveoli (Stain H&E, X10).

Fig (7) High power of the previous picture showing inflammatory cells aggregation mainly neutrophiles inside acini and destruction of epithelium lining (Stain H&E, X40).

Fig (8) Supramammary lymph node affected by acute *S. agalactiae* infection showing suppurative lymphadinitis, vasculitis and interstitial edema (Stain H&E, X10).
Fig (9) Mammary gland affected by *E. coli* mastitis showing acute suppurative mastitis, strands of fibrin with cellular debris and caseated milk inside alveoli and infiltration of inflammatory cells mainly neutrophils and macrophages with destruction of epithelium lining (Stain H&E, X 10).

Fig (10) Supramammary lymph node affected by acute coliform infection showing suppurative lymphadinitis, severe haemorrhage and interstitial edema (Stain H&E, X 10).

Fig (11) *Helminthosporium* and *Stemphillium* isolated from mammary gland (from culture, X 100).

Fig (12) Supramammary lymph node affected by acute fungal infection showing suppurative lymphadinitis, congestion and interstitial edema (Stain H&E, X 10).
Fig (13) Normal control monolayer MDBK cells. (Stain carbon fuchsin, X4)

Fig (14) Monolayer MDBK cells inoculated with *S.aureus* after 6 h showing marked tissue damage. (Stain crystal violet, X 4), * attachment of *S.aureus* to both damaged & living cells (Stain crystal violet, X 40).

Fig (15) Monolayer MDBK cells inoculated with *E.coli* after 6 h showing the cytopathic effect became more severe as evidenced by extensive areas of erosion . (Stain carbon fuchsin, X 4), * loss of attachment between cells and colonies of *E. coli* (Stain carbon fuchsin, X 40).

Fig (16) Monolayer MDBK cells inoculated with *S. agalactiae* after 6h showing less tissue damages. (Stain crystal violet, X4), * S. agalactiae colonies (Stain crystal violet, X 20).
DISCUSSION

During infection of the mammary glands, the tissue damage can initially be caused by bacteria and their products. Certain bacteria produce toxins that destroy cell membranes and damage milk-producing tissue, whereas other bacteria are able to invade and multiply within the epithelial cells before causing cell death. In addition, mastitis is characterized by an influx of somatic cells, primarily neutrophils, which cause breakdown of the blood-milk barrier and damage mammary epithelium (Zhao and Lacasse, 2008).

In this study, bacteriological investigation were in agreement with Bansal et al. (1990); Seham and Nafady (1995); Benites et al. (2002) and Moroni et al. (2006). Our results clarified that the highest incidence of isolated bacteria was *S. aureus* and CNS while the lowest one was *Citrobacter freundii*. These findings are concomitant with those given by Bansal et al. (1990) and Seham and Nafady (1995). While, El-Rashidy et al. (1996) stated that *E. coli* was the highest incidence. The increased incidence of staphylococci may be attributed to the indiscriminate use of antibiotics which results in the emergence of more resistant strains. Secondly, these organisms survive better in the environment and are widely distributed at different body sites such as teat apices (Bansal et al., 1990).

In the present study, gross and histopathological examination of the mammary glands of acute and chronic *S. aureus* mastitic cases were in accordance with that reported by Shibahara and Nakamura (1999) and Derabin and Kurlaev (2000).

β toxins of *S. aureus* damage mammary secretory epithelial cells and increase adherence and invasion to tissue as well as enhance the proliferation in the affected tissue (Saad et al., 2000). Radostitis et al. (2000) explain the variable severity of acute mastitis, staphylococcus can propagate within the glandular tissue which blocked by the colonized bacteria together with inflammatory exudate, hence aggravation of the condition can take place and severe involvement of the obstructed area supervenes.

Jones et al. (1997) explain the frequent presence of corpora amylacea, by time, the exudative stage subsides and macrophages, lymphocytes and fibroblasts dominate the residual inflammatory process. At this stage, affected acini and ducts begin to involutes, cease secretion, and become distended by luminal secretion and debris.

Our findings in lymph nodes affections in case of *S. aureus*
were coincided with, Seham and Nafady (1995) and El-Mahdy et al. (2002). The severe lymphoid depletion observed point to the severity and virulence of organism (Seham and Nafady, 1995). Necrosis and suppuration of lymph nodes could be attributed to leukocidin generated from staphylococci that kill neutrophils and macrophages (Quinn et al., 2002). El-Mahdy et al. (2002) who pointed out that the occurrence of haemorrhages in lymph nodes of cattle is not attributed to the changes occurred during slaughtering but mostly related to bacterial infection.

Benites et al. (2002) said that CNS strains have received little attention, where it induces subclinical mastitis. However the degree of inflammation of the udder is less than that associated in other pathogens. CNS may produce several extracellular components, but their contribution to pathogenicity is not clearly understood. Attention has been paid to haemolysin produced by CNS which may be cytotoxic and promoting tissue necrosis. The recorded results of CNS mastitis were similar to findings given by Benites et al. (2002) and Winter et al. (2003).

*S. agalactiae* persist shortly in the environment although it can survive for long period only within the gland. Once the organism gains entrance into the gland, it is very persistent and it does not leave the gland unless the animal is treated. However streptococci other than *S. agalactiae* can survive for long periods outside the mammary gland, and can colonize intact skin (Refai, 1988 and McGavin et al., 2001).

Our results in Streptococcus mastitis were similar to findings given by Thomas et al. (1994) and Seham and Nafady (1995). Thomas et al. (1994) explained the process of fibrosis, during acute flares of mastitis caused by *S. agalactiae* was due to the response to chemotactic factors released either from the organism itself or cells damaged by it, the surrounding connective tissue becomes markedly edematous with extravasations of large numbers of neutrophils into the surrounding connective tissue and alveoli. Increasing amounts of fibrous connective tissue begin to be deposited in the periglandular and periductal connective tissue and obliterates the lumens of ducts and acini.

The same results of lymph nodes obtained by El-Mahdy et al. (2002) who stated that the observed depletion could be attributed to the toxiginicty of the organism.

Coliform organisms are a relatively uncommon cause of mastitis in cattle; the disease is usually sporadic (Refai, 1988). Similar to our findings in Coliform
mastitis were obtained by Hill et al. (1984); Dopfer et al. (2001) and Hogan and Larry (2003). Our results disagree those given by Radostitis et al. (2000) who stated that the progressive pathological changes in E. coli mastitis are most marked in the epithelium of the teat and lactiferous sinuses.

Hogan and Larry (2003) reported that tissue changes in E. coli mastitis that dominates the acute response as the result of endotoxic injury to the microvasculature of the alveolar walls and mammary interstitium. Provoke massive neutrophils emigration to clean the gland of organisms within few days.

Schalm (1977) have explained the chronic reaction that develops as follows; when the initial inflammatory response fails to complete clear the gland of all coliform organisms. The tissue reaction is then formed of fibroblastic activity. Hyperplasic activity in chronic cases could be considered as a trial for repair of the damaged epithelial lining. The main blood vessels of the affected lobules showed thickening of their tunica media. In this respect McGavin et al. (2001) stated that coliform mastitis often develop chronic mastitis.

The incidence of mycotic infection of the udder was not sufficiently assessed in lactating cattle although it is becoming prevalent. The present study indicated that apparently healthy udders may harbour pathogenic fungi, the matter which has a great epidemiological importance as it may cause severe harm for milk consumers. Our findings were similar to those reported by Bansal et al. (1990); El-Rashidy et al. (1996). The increased incidence of mycotic mastitis may be attributed to prolonged and indiscriminate use of antibiotics and steroids in udder therapy, non-availability of suitable drugs for treatment of mycotic mastitis and persistence of fungal organisms in dairy environments (Bansal et al., 1990).

The gross and microscopical changes observed in the present work were similar to those described by Costa et al. (1993); El-Naggar et al. (1997) and Preze et al. (1998). In this respect El-Naggar et al, 1997 reported that the major defense against hyphae was polymorphonuclear cells and macrophages which were essential for killing spores.

From our study, comparison with mammary pathogens, indicated that E. coli invaded MDBK cells less efficiently than S. aureus and more efficiently than S. agalactiae. These findings came in accordance with those given by Semjen and Galfi (1990); Saad et al. (2000) and Lorena et al. (2005). The adherence of microorganisms to epithelial cells is an
important part of the pathogenesis of various infectious diseases. Attachment to cell surfaces affects the ability of microorganisms to survive and multiply, especially in lumina that undergo periodic flushing actions (Dogan et al., 2006). The interaction between bacterium and host cell involves the binding of surface-associated bacterial ligands to surface receptors on the host cell. The nature of the ligand may vary, streptococci and S. aureus utilize the lipoteichoic acid component of their external wall as ligand but the Enterobacteriaceae have the fimbriae which serve as the ligand in the attaching process (Semjen and Galfi, 1990).

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الميكروبات المسببة لالتهاب الضرع وعلاقتها بالتغيرات histopathological
لأنسجة الضرع وغدد اللبمفافيا في الجاموس
عبر التمثول*، هناء عصفور**
قسم البياتولوجيا - معهد بحوث التناسليات الحيوانية بالهرم
قسم الضرع والنتائج - معهد بحوث التناسليات الحيوانية بالهرم

الملخص العربي

تم تجميع 112 عينة من أنسجة الضرع والغدد اللمفاوية المصاحبة لها وفحصها
ميكيوبولوجيا وهستوباثولوجيا. وشملت الميكروبات المعزولة من الميكروب العنقودي
الذهبي و الميكروب العنقودي سالب التجلج و الميكروب السبهي بانواعه و الميكروب
القولوني بانواعه. وقد نبض بالفحص histopathological وجود الالتهابات حادة ومزمنة في
أنسجة الضرع. أما الغدد اللمفاوية في حالات الالتهابات البكتيرية؛ فقد أظهر الفحص
الهستوباثولوجي وجود بقع نزفية وارتشاحات والالتهابات صغيرة. وقد تم عزل انواع
مختلفة من الفطريات من أنسجة الضرع والغدد اللمفاوية المصاحبة لها منها:
لاسيرجيالس والكلاداوسوريم والفيتالوفورا والبسينيل بانواعهم المختلفة. وقد كان الفحص
المجهري في حالات الالتهاب القلاب الحاد يتميز زيادة تجميع الخلايا الالتهابية مع
تكسير في الأنسجة المفرزة للبن. بينما في حالات الاصابة المزمنة بالفطريات فقد وجد
تجمع خلايا ليمفاوية وبلعمية وخلايا عمالقه بحجم مختلفه. وفي هذا العمل تم استخدام
Madin Darby Bovine Kidney cell line (MDBK)
لاختيار مدى تأثير بعض الميكروبات المعزولة وخصوصا الميكروب
العنقودي الذهبي والميكروب السبهي والميكروب القولوني على هذه الخلايا خلال
24 ساعه عند درجه حراره 37 grad. ومن هذه التجربه وجد ان الميكروب العنقودي الذهبي له
التأثير الآقلي على حيويه الخلايا الاحادية ثم الميكروب القولوني واقله تأثيرا الميكروب
السبهي.

المحموم:

أ.د. نبيهه رمضان حسن
أ.د. عادل بكير خلوفى