Bacteriological and pathological studies on kidney affections of slaughtered buffaloes at Kaluobia Governorate

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SUMMARY

Microbiological and histopathological studies were carried out on thirty apparently diseased kidneys collected from slaughtered adult female buffaloes at Banha abattoir – Kaluobia Governorate. Bacteriological investigations revealed that seven different bacteria of both Gram positive and Gram negative bacteria were recovered from the affected kidneys namely: *Escherichia coli* (23.3%), *Staphylococcus aureus* (13.3%), *Pseudomonas aeruginosa* (10.0%), *Streptococcus* SPP. (3.3%), *Enterococcus faecalis* (3.3%), *Corynebacterium* SPP. (3.3%) and *Klebsiella pneumoniae* (3.3%). Also, 10% of the examined samples revealed more than one pathogen (mixed infection). Antibiogram was applied upon the isolated bacterial pathogens.

Pathological studies revealed that 70% of the investigated kidneys showed the histopathological picture of pyelonephritis due to infection with various type of bacterial pathogens either in single or mixed form. On the other hand 30% of the examined kidneys exhibited massive degeneration & sloughing of the renal tubular epithelium with formation of epithelial casts (tubulo-nephrosis).

INTRODUCTION

Pyelonephritis frequently occurs in cattle, buffaloes and to a lesser extent in sheep. In large ruminant, it is a polybacterial infection that invariably include, *Corynebacterium renale*, *C. pseudotuberculosis*, *C. cystitidis*, *Escherichia coli*, *Arcanobacterium pyogenes*, *Staphylococcus aureus*, *Streptococcus*, *Enterococcus*, *Proteus*, *Klebsiella* and *pseudomonas*.
species (Timonedy et al., 1988 a; Maxie and Prescott, 1993; Sheldon, 1995 and Van Metre and Divers, 2002).

Pathogenesis of pyelonephritis mainly depend on the abnormal reflux of bacterial contaminated urine from the lower tract to the renal pelvis and collecting tubules (Vesicoureteral reflux) as in urethral obstruction and cystitis (William and McDonald, 1995 and Lucky, 2003).

The objective of this study was to survey and identify the bacterial causative agent which were associated with buffaloes kidney affection and to describe the pathological findings encountered in such cases, in addition to the in vitro assay of antibiotic versus the various isolates.

MATERIAL AND METHODS

The present work was conducted on thirty apparently diseased kidneys which were collected from slaughtered adult female buffaloes at Banha abattoir. One portion of each sample was taken under aseptic condition and placed in a sterile plastic container and transported in an ice box to the laboratory for bacteriological investigations.

Bacteriological examination:
The surface of each sample was touched by hot spatula and opened under complete aseptic precaution. A swab of each sample was directly cultured onto nutrient broth and incubated at 37°C for 24 hours and then an inoculum was cultivated on different selective media including agars “Difico” nutrient agar, 5% sheep blood agar, MacConkey agar, S.S. agar and XLD agar.

All inoculated plates were incubated aerobically at 37°C for 24 – 48 hrs. The suspected colonies were examined culturally, morphologically as well as biochemically as described by Cruickshank et al. (1975); Baily and Scott (1994) and Quinn et al. (1994).

Antibiogram study:
Drug sensitivity test of all isolated bacteria was done using standard disc technique according to Boone and Castenholz (2001) against eight different chemo therapeutic agents. The BioMerieux discs which were used for the in vivo assay namely erythromycin (15 μg), Garamycin (30 μg), Kanamycin (30 μg), neomycin (30 μg), oxytetracycline (10 μg), spectinomycin (10 μg), chloramphimicol (30 μg) and ampicillin (10 μg).

Histopathological studies:
Selected portions from each affected kidney were fixed in 10% neutral buffered formalin solution and processed by the standard paraffin embedding technique. Tissue specimens were cut at 5 micron &
stained with Hematoxlin & Eosin and examined microscopically (Bancroft et al., 1994).

RESULTS

Bacteriological findings:

Cultures of kidney samples as well as biochemical identification of isolates from all samples resulted in the detection of seven different species of both Gram positive and Gram negative bacteria. *Escherichia coli* 7 (23.3%), *Staphylococcus aureus* 4 (13.3%), *Pseudomonas aeruginosa* 3 (10.0%), *Streptococcus* SPP. 1 (3.3%), *Enterococcus faecalis* 1 (3.3%), *Corynebacterium* SPP. 1 (3.3%) and *Klebseilla pneumoniae* 1 (3.3%) were the isolated bacterial strains from the examined buffalo’s kidneys (Table 1).

These isolates were recovered from 21 samples out of 30 examined kidney samples (70%). Three kidney samples revealed double isolates, two of them revealed both *E. coli* and *Staphylococcus aureus* 2 (6.6%) while the third one had *E. coli* and *Corynebacterium* SPP. 1 (3.3%).

Antimicrobial sensitivity test revealed that garamycin (30 μg) was the most sensitive antibiotic against all isolated microorganisms and ampicillin was the drug of choice for all isolates except *E. coli* and *Klebseilla* did not respond to it.

Table (1): The number and parentage of isolated microorganisms from kidney samples of buffaloes.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Number of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single infection:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- <em>E. coli</em></td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>- <em>Staphylococcus aureus</em></td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>- <em>Pseudomonas aeruginosa</em></td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td>- <em>Streptococcus</em> SPP.</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>- <em>Enterococcus faecalis</em></td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>- <em>Corynebacterium</em> SPP.</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>- <em>Klebseilla pneumoniae</em></td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Mixed infection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- <em>E. coli</em> + <em>Staph. aureus</em></td>
<td>2</td>
<td>6.6</td>
</tr>
<tr>
<td>- <em>E. coli</em> + <em>Corynebacterium</em> SPP.</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>21</td>
<td>70</td>
</tr>
</tbody>
</table>
Pathological findings:

On necropsy, some kidneys appeared swollen and capsules were thickened and tightly adherent to the underlying cortex. The surface of which was mottled red & fawn. Cut section showed multiple white striations throughout the cortex & medulla was interspersed with areas of hemorrhage & abscess formation in the kidney. On the other hand, some kidneys were pale & greatly enlarged. Capsule stripped readily to reveal smooth unpitted surface containing circular red or sometimes yellow spots of 1 – 2 mm in diameter. The cortex was tough when cut & the spots noted superficially throughout their entire depth.

Microscopically, multiple severe necrotic foci involving the glomeruli & tubule were observed. There were proliferating glomerulonephritis including mononuclear invasion of the renal tissue with inflammatory cells (Figs., 1 & 2), which consisting mostly of polymorphs and mononuclear cells.

Some glomerular tuft showed marked increase in cellularity as a result of proliferation of endothelial cells and infiltration of polymorphonuclear cells (Fig., 3). On the other side, some glomeruli were atrophied (Fig., 4). Some glomerular tuft showed proliferation associated with hyalinization of the wall of the glomerular capillaries (Fig., 5).

The tubular lumen contained casts which consists of inflammatory cells, desquamated and degenerated epithelial cells, especially noticed in the medulla (Figs, 6 and 7). Cystic dilatation of the renal tubules with proliferation of the fibrous connective tissue were noticed (Figs., 8).

The collecting tubules surrounding renal pelvis in the renal medulla undergo degenerative necrobiotic changes associated with severe congestion of the interstitial blood vessels (Fig., 9).

Some kidneys showed tubulonephrosis where massive degeneration & sloughing of the renal tubular epithelium as well as sloughed degenerated epithelial cells casts were observed in the renal tubules (Fig., 10). In some cases, the renal tubular epithelium undergoes necrosis with information of intraluminal casts (Fig., 11). Vacuolar degeneration of glomerular tuft capillaries were observed (Figs., 11 & 12).
Table (2): Antibiogram of the isolated microorganisms.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Erythromycin 15 µg</th>
<th>Chloramphenicol 30 µg</th>
<th>Kanamycin 30 µg</th>
<th>Neomycin 30 µg</th>
<th>Garamycin 30 µg</th>
<th>Oxytetracyline 30 µg</th>
<th>Ampicillin 10 µg</th>
<th>Spectinomycin 20 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Streptococcus SPP.</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Corynebacterium SPP.</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Klebseilla pneumoniae</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>
Fig. (1): Kidney revealing intraluminal hyaline casts with dense interstitial mononuclear cells infiltration (H & E x 200).

Fig. (2): Kidney showing interstitial infiltration of mononuclear cells (H & E x 400).

Fig. (3): Kidney showing proliferation of capillary tuft (H & E x 400).

Fig. (4): Kidney showing extraglomerular edema with shrinkage of glomerular tuft (H & E x 400).
Fig. (5): Kidney exhibiting congestion and proliferation of the glomerular tuft (H & E x 400).

Fig. (6): Congested kidney medulla showing vacuolation and destruction of tubular epithelium (H & E x 200).

Fig. (7): Kidney medulla revealing hyaline casts (H & E x 400).

Fig. (8): Kidney showing cystic dilatation of the renal tubules, proliferation of the fibrous connective tissue (H & E x 100).
Fig. (9): Kidney showing congestion of renal capillaries as well as tubular calcification (H & E x 400).

Fig. (10): Kidney revealing destruction of tubular epithelium (H & E x 200).

Fig. (11): Renal medulla showing tubular edema and epithelial casts (H & E x 100).

Fig. (12): Kidney revealing vacuolation of the glomerular tuft (H & E x 200).
DISCUSSION

Pyelonephritis in ruminants can be a herd problem (Maxie and Prescott, 1993). Nowadays, this is relatively in excess as reported by many veterinarians working in the field of meat inspection, that is because of subjecting the animals to different types of rations in addition to over spreading of pollution.

In the present study, affected bovine kidneys were enlarged and the capsule was easily stripped. The surfaces of which was mottled with circular red or sometimes yellow spots. Braun et al (2008) observed similar findings in kidneys of 17 cattle suffered from pyelonephritis as a result of infection with Corynbacterium renale, Arcanobacter pyogenes and Escherichia coli.

In the present work most common isolates observed were E. coli (23.3%) and Staphylococcus aureus (13.3%). E. coli is a normal inhabitant of lower intestine & is abundant in faeces and in the environment (Timonedy et al., 1988 b). E. coli also, may be a common pathogen involved in urinary tract infection (Rebhum et al., 1989). Rosenbaum et al. (2005) reported that E. coli, Arcanobacter pyogenes, Coryn. renale and Coryn. cystitidis were isolated from 21 rejected kidneys of dairy cows suffered from pyelonephritis in slaughter house survey.

Streptococcus species and Entercococcus faealis (3.3%) are also, opportunists & have been associated with pyelonephritis as recorded in cows (Maxie and Prescott, 1993).

Corynebacterium species (3.3%) is an obligate pathogen of the urinary mucosa. Hirbrunner et al. (1996) have isolated Coryn. renale from cow suffered from unilateral pyelonephritis.

Pseudomonas aeruginosa (10.0%) and Klebseilla penmoniae (3.3%) were recovered in the present study and was considered as a common agent of pyelonephritis in buffaloes as recorded by Timonedy et al. (1988 c).

Antimicrobial sensitivity test revealed that garamycin (30 μg) and Ampicillin (10 μg) were the antibiotic of choice for combating pyelonepritis in the present study and come parallel with those recorded by Fowler (1998).

In this study, the pathological picture of 21 cases of pyelonephritis showed multiple necrotic areas involving the glomeruli and renal tubules. Invasion of the renal parenchyma with inflammatory cells consisting mostly of polymorpho and mononuclear cells. The most characteristic findings were observed in the collecting tubules
surrounding the renal pelvis that undergo degenerative and necrobiotic changes, especially in animals subjected to mixed infection.

Recently, it was shown that neutrophils have the capacity to synthesize & release several cytokines either constitutively or following stimulation. These cytokines induced recruitment and activation of mononuclear inflammatory cells and explaining the presence of the observed renal lesions (Cassatella, 1995). Similar lesions, also, noted in the renal tissues of animal affected by pyelonephritis (Young et al. 1981; Jones et al., 1997).

Some kidneys showed tubulonephrosis where massive degeneration and desquamation of the renal tubular epithelium forming epithelial casts within the renal tubules. This pathological picture was also given in some cases mentioned by Angus et al., (1989); Confer and Panciera (1995) and Bucci et al. (1998).

From the present study, it was concluded that *E. coli*, *Staphylococci* and *Pseudomonas* are the most common pathogens which cause kidney problem in buffaloes in Kalubia Governorate. Recommendation for treating and controlling the clinical cases with the drug of choice based on the isolation of the causative organism and on the antibiogram study.

REFERENCES


دراسة بكتيرولوجية وبياثولوجية عن بعض إصابات الكلى في الجاموس

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قسم المعامل الفرعية في الباثولوجيا - معهد بحوث صحة الحيوان
قسم البكتيرولوجيا - معهد بحوث صحة الحيوان

الملخص العربي

أجرت هذه الدراسة على عدد ٣٠ حالة من كلى الجاموس، تم تجميعها من محافظات القلوبية. وبعد أجراء الفحوص البكتيرولوجية والبياثولوجية، وجد أن هناك أنواع مختلفة من البكتيريا سالبة الجرام والموجبة للجرام. وان ميكروب الاشريشيا كولاري كان يمثل ١٣٠٪، و ميكروب أستاف أوريس ٧٦٪، وميكروب السيدموناس ١٠٪، وميكروب سرلينوكونس ٣٣٪، وميكروب الأنغروكونس ٣٣٪، وميكروب كورني باكتيرم ٣٣٪، وميكروب الكلبسيلا نيموني ٣٣٪. وقد وجد أن ١٠٪ من العينات بيئة أكثر من ميكروب واحد وتم عملية اختبارات الحساسية البكتيرية المعزولة بالفحص البياثولوجى للعينات. وأوضح النتائج أن ٥٪ من أنتهاكات كلية نتيجة الأشعة بأنواع من البكتيريا السالية والموجبة للجرام، وكان ٣٠٪ من العينات وجد بها استجابة وتكسير في بعض خلايا الأنابيب البولية مع ظهور بعض الأسطوانات الكلوية داخل الأنابيب البولية.

المحكون:

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