

STUDIES ON RENAL BACTERIAL AFFECTIONS IN SHEEP IN MATROUH GOVERNORATE

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ABSTRACT

Urine and tissues samples were collected for bacteriological examination from 50 apparently diseased sheep of different ages and sexes which were clinically suspected suffering from renal infection and from slaughtered animals. We were collected samples from many locations at Matrouh Governorate and sent for laboratory. Post mortem examination of the renal system of affected cases revealed different investigations. kidneys appeared swollen, its surface was mottled red. Some kidneys were pale and greatly enlarged. Cut section of the kidney showed areas of hemorrhage and abscess formation. The results indicated that 80% (40) sheep were positive for bacterial infection however 10 show negative results of bacterial renal infection. Bacteriological investigations revealed that the isolated organisms were: *Escherichia coli* (20%), *Staphylococcus aureus* (25%), *Pseudomonas aeruginosa* (5.0%), *Streptococcus spp.* (7.5%), *Enterococcus faecalis* (7.5%), *Corynebacterium spp.* (17.5%), *Klebsiella pneumoniae* (2.5%). and *Listeria monocytogene* (2.5%). Moreover, mixed infection found in 12.5% of the examined samples. A total of eight isolates of E coli from renal examined samples were serologically positive to O111, O104, O26, O113, O91, O103 and O126. PCR results showed that two strains for *Listeria* isolates were *Listeria monocytogenes* and some of them have InI B gene.

Antibiogram was applied upon the isolated bacterial pathogens and found that Garamycine was the drug of choice for treatment of infected animals.

Keywords: Urine, tissue, diseased, age, sex der.

INTRODUCTION

An infection of urinary tract is the most highly bacterial infections worldwide. Inflammation of the kidneys were the most findings in sheep. El-Mashad Bancroft *et al.*, 2019 and (Sjo"lund *et al.*, 2020). Animal productivity is reduced both quantitatively and qualitatively as a result of the urinary system, which results in

severe economic losses. (Ahmed *et al.*, 2020). Reviewing the diseases of sheep in the Egyptian literature 30 years one can realize that urinary system diseases constituted a great problem especially urethra and urinary bladder, however the kidneys did not receive much attention. (Nehal, 2004) and (Ali, and Khalid. (2017).

Inflammation of mostly parts of kidneys was named as. Pyelonephritis it is occasionally affected sheep while isolated firstly a bovine diseases (Radostits *et al.*, 2007). Pyelonephritis's pathogenesis is mainly resuled from the bacterial abnormal

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reflex of contaminated urine across all urinary tract from lower part to the renal pelvis and collecting tubules (Vesicoureteral reflux) as in urethral obstruction and cystitis (Lucky, 2003). The most significant bacteria infected are *E. coli*, *K. pneumoniae*, *Actinomyces pyogenes*, *Staph. Aureus*, *Corynebacterium renale* and *Proteus* (Radostits *et al.*, 2007). Enteropathogenic *E. coli* (EPEC) is a subset of pathogenic *E. coli* can cause infections in sheep (Cheila *et al.*, 2011). Several *E. coli* serogroups were isolated somewhat differ in each study (Cid *et al.*, 2001, Wani *et al.*, 2004 and Cecilie *et al.*, 2013).

An infections with *Proteus*, *Klebsiella*, *Citrobacter*, *Enterobacter*, and *Pseudomonas* species and *Staphylococcus saprophyticus*, and streptococci causes Cystitis in ruminant. *Escherichia coli*, is the most common agent for infectious cystitis (Echols *et al.*, 1999), Pyelonephritis cystitis, and urethritis in sheep and cattle are most usually brought on by an ascending urinary tract infection (UTI) with *Corynebacterium renale* or *Escherichia coli*. *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., While as *Arcanobacterium pyogenes*, *Corynebacterium pseudotuberculosis*, and other *C. renale* group members are less frequent causal organisms. Although it is far less common, bacteraemia with agents like *Salmonella* species, *Arcanobacterium pyogenes*, or in small ruminants, *Corynebacterium pseudotuberculosis* can cause renal infection via the hematogenous pathway (suppurative embolic nephritis) (Yeruham *et al.*, 2006, Radostits *et al.*, 2007; Smith, 2009; Abdel-Baset *et al.*, 2019).

These bacteria not only show signs of a urinary tract infection but were isolated from apparently healthy cattle. Additionally, they have pathogenesis-predisposing variables such urolithiasis, problematic or late pregnancies, prostatic hypertrophy, paralysis of the bladder, and urethral abnormalities.

(Normohammadzadeh *et al.*, 2003; Yeruham *et al.*, 2006 Radostits *et al.*, 2007; Smith, 2009). Shedding of mature epithelial cells with attached bacteria through normal micturition is the urinary tract defense mechanism against bacterial infection and colonization. The amount and severity of the bacterial infection, the existence of urogenital trauma or aberrant vulvar conformation, obstetric manipulation, bladder catheterization, and urine retention are all factors in ascending UTI Ali, and Khalid. (2017) Ahmed *et al.*, 2013 and Smith, 2009). Enterotoxigenic *Escherichia coli* are considered the most causative agents of renal affection in sheep. Enteropathogenic *Escherichia coli* (EPEC) is a subset of pathogenic *E. coli* (Cheila *et al.*, 2011). Several *E. coli* serogroups were isolated somewhat differ in each study (Cid *et al.*, 2001, Wani *et al.*, 2004 and Cecilie *et al.*, 2013). *Salmonella* is a main reason of renal affection diseases in sheep and goat in the world. It has been isolated from the feces and urine of sheep and goats by ratio of 0.7% (Molla *et al.*, 2006; Moyaert *et al.*, 2019 Mahouz *et al.*, 2015): Elgumaa *et al.* (2017) and Cheila *et al.*, 2011).

AIM OF WORK

The current study is carried out to employ antibiogram diffusion technique to isolate and identify bacterial agents that affected urinary tract in sheep as well as determine the best drug to treat them.

MATERIALS AND METHODS

1-Animals:

This study was carried out on: 50 sheep of different ages and sex from different regions in Matrouh Governorate suspected clinically of renal affection from frequent intermittent and painful urination, changed urine color and odor also sometimes found rise of body temperature with depression, loss of appetite and general weakness. Post mortem examination of kidneys in slaughtered or dead animals was done.

2-Samples:

For bacteriological examination, urine sample was taken under complete aseptic condition directly by urinary catheter or by discard the first drops of urine and take the middle from living animals, in clean and sterile Mc.Carteny bottles and after centrifugation of the urine ,we examined the sediment bacteriological in the laboratory.. Another samples were collected under aseptic condition from slaughtered animals including whole kidney and urinary bladder after tying the urinary bladder and put in sterile plastic container. We were preserved all samples in ice boxes and immediately transported to the lab for bacteriological analysis. Foreyt (2001)

3-Bacteriological examination:

For urine samples:

Centrifugation of the samples in centrifuge at 2000 r.p.m. for 10-15 m. to bright sediment (Boöll and Gründer, 1981 and Leng, 1995) then inoculated the sediment with a platinum loop into blood and McConkey plates and incubated at 37°C for 72 hrs. We examined growing colonies by naked eye for size, shape, and zone of haemolysis.

Microbiological analysis for *Listeria* isolation:

Fraser broth (Merck) was used for enrichment and the selective agar media ALOA®, Merck agar (Merck), OXFORD were used for selective plating.

For isolation and identification of *L. monocytogenes* we was used ISO 11290 method in this study as described by Becker *et al.* (2006).

For tissue samples:

The surface of each sample was touched by hot spatula and opened under complete aseptic conditions by scalpel. Each sample was immediately cultured on nutrient broth for 24 hours at 37 °C, and then an inoculum was grown on various selective medium, such as nutrient agar, 5% sheep blood agar, and MacConkey agar. All inoculated plates

were incubated aerobically at 37°C for 24-48 hrs. The suspected colonies were identified cultural, morphological and biochemical characters according to [Baily and Scott (1994) and Quinn *et al.* (1994)].

4-Microscopical examination:

Each colony's smear was removed, spread out on a dry, clean slide, stained with Gram stain to show gram positive and gram negative bacteria, and then examined under a microscope to look at the morphology of the microbes.

5-Biochemical characteristics:

Specific tests in which the isolates could be identified including coagulase test, haemolysin on blood agar plates and gelatin liquefaction (Crookshank 2007).

Serological identification:

The isolated *E. coli* strains were sent for clinical microbiology units in Benha University Faculty of Veterinary Medicine-Food Analysis Center for serological identification

6-Serological identification of *E. coli*

The isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic antisera sets (DENKA SEIKEN Co., Japan) for detection of the Enteropathogenic types.

Genotypic detection of isolated *L.monocytogenes* using polymerase chain reaction (PCR)

Three sets of primers was used in PCR to detect genes of *L.monocytogenes* strains and 10 virulence genes of *L.monocytogenes*. The internalin B (inlB) was virulence genes Kumar *et al.*, 2015.

Analysis of the PCR Products.

PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was put in each gel slot. A

Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) using determine the fragment sizes. A gel documentation system (Alpha Innotech, Biometra) was using for photographed the gel and computer software was using for analyzing the data. From Metabion German, Oligonucleotide Primer. Primers used have been supplied.

PCR amplification: In a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan) primers were utilized, 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. In an Applied biosystem 2720 thermal cycler reaction was performed,

7-Antibiogram study:

All isolated bacteria were tested for antimicrobial sensitivity using the conventional disc technique (Boone and Castenholz 2001). Eight different chemo therapeutic agents (BioMerieux discs) were used. The discs 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), chloramphicol (30 µg) and ampicillin (10 µg).

RESULTS

Post mortum examination of the renal system of affected animals showed that some kidneys appeared swollen, the capsules were thickened and tightly adherent to the underlying cortex. The surface of which was mottled red and fawn. Cut section showed multiple white striations throughout the cortex and medulla was interspersed with areas of hemorrhage and abscess formation in the kidney. On the other hand, some kidneys were pale and greatly enlarged. The cortex of kidneys was rough and strong when cut. There were spots on the surface throughout their entire depth.

The bacteriological examinations of suspected cases of urinary tract affections in sheep revealed that 40 (80%) cases was positive for renal tract infections the number of females affected are more than males. The percentage of infected females 62.5% from all positive cases while that of males 37.5% from positive cases which constitute 80% from all sheep animals. There was no significant difference between their ages.

Table 1: Biological sex influences susceptibility to bacterial infection in examined cases:

Total number (50)		Females		Males	
+	-	+	-	+	-
40	10 (20%)	25	3	15	7
(80%)					

The bacteriological findings:

Identification of isolates from all samples resulted in the detection of 40 bacterial isolates. The isolates constituted 7 genera from different species of both Gram positive and Gram negative bacteria. The following strains were recognised as the isolated ones:

Streptococcus spp. 3 (7.5%), *Enterococcus faecalis* 3 (7.5%), *Corynebacterium renal* 7 (17.5%), *Escherichia coli* 8 (20%), *Staphylococcus aureus* 10 (25%), *Pseudomonas aeruginosa* 2 (5%), *Streptococcus* spp. 3 (7.5%), and *Klebseilla pneumoniae* 1 (5%), *Listeria monocytogene* 7 (12.5%) and mixed infection 1 (5%) Out of the 30 samples that were tested, these isolates were found in 21 samples with positive results (about 70%). Five samples exhibited two isolates each; of them (6.7%) showed *Listeria monocytogene* while 3 isolates the mixed bacteria (3.3%) included *Corynebacterium renal* and *Streptococcus* spp.

Table (2): Incidence of the isolated micro-organisms from sheep samples of renal affection:

Micro organisms	Positive samples	
	NO.	%
Single infection:		
<i>Staph. Aureus</i>	10	25
<i>Corynebacterium renal</i>	5	17.5
<i>Streptococcus spp.</i>	3	7.5
<i>E.coli</i>	8	20
<i>Pseudomonas aeruginosa</i>	2	5
<i>Enterococcus faecalis</i>	3	7.5
<i>Klebsella pneumonia</i>	1	2.5
<i>Listeria monocytogene</i>	1	2.5
<i>Coryn. renal and streptococcus</i>	7	12.5
Total	40	100

Antimicrobial sensitivity test revealed that garamycin (30µg) was the most effective antibiotic against most isolated microorganisms of *Staph. aureus* and *E. coli*. However, oxytetracycline (30µg) was the drug of choice for *Corynebacterium renal*.

Table (3): Antibiogram test of the isolated strains

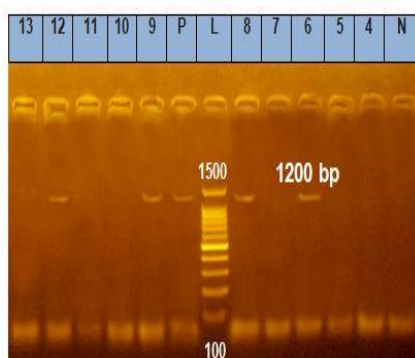
Bacterial spp. Antibiotic discs	<i>Staph. aureus</i> (10)	<i>Coryn.renl</i> (7)	<i>Strept. spp.</i> (3)	<i>E.coli</i> (8)	<i>Pseudomonas aeruginosa</i> (2)	<i>Entero. Faecalis</i> (3)	<i>Klebsella pneumoniae</i> (2)
Antibiotic							
Erythromycin (15µg) S %	4 46%	3 46%	2 50%	1 10%	2 80%	1 30%	0 9%
Chloramphenicol (30µg) S %	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Kanamycin(30µg) S %	1 6%	1 10%	1 2%	4 46%	1 10%	0 0	1 60%
Neomycin (30µg) S %	0 0	0 0	0 0	1 10%	1 20%	0 0	0 0
Garamycin 30µg) S %	8 80%	8 76%	2 60%	6 80%	2 84%	2 50%	1 65%
Ampecillin(10µg) S %	2 18%	6 80%	2 50%	1 5%	0 0	2 75%	0 9%
Oxytetracycline (30µg) S %	7 73%	2 25%	3 90%	3 35%	1 67%	2 60%	1 37%
Spectinomycin (20µg) S %	1 70%	3 40%	2 50%	4 50%	1 22%	1 27%	0.2 10%

S: sensitive %: percent of sensitivity

Table (2): Serological identification of *E. coli* strain from fecal samples of diseased sheep

Identified strains	No of total isolates	Male	Fmals
<i>E. coli</i> O111 : H2 EPEC	2 isolates	1isolates	1 isolates
<i>E. coli</i> O104: H4 EPEC	1 isolates	3 isolates	----
<i>E. coli</i> O26: H11 EPEC	1 isolates	1 isolates	----
<i>E. coli</i> O113 : H21 EPEC	1 isolates	=====	1 isolate
<i>E. coli</i> O91 EPEC	1 isolate	1 isolate -	=====
<i>E. coli</i> O103: H2 EPEC	1 isolate	==	1 isolate
<i>E. coli</i> O126 EPEC	1 isolate	===	1 isolate

Fig. (2): In IB genes. Lane L:100-1000 bp
Ladder. Neg: Negative control.
Pos: Positive control at (174bp).
Lanes 1 to 12



DISCUSSION

Renal system is considered to be one of the four main channels by which living animals eliminated its metabolic waste products. One of the vital and important organs of these are kidneys which posses a great importance for the stability of internal environment of the body it is well known also that if both kidneys are destroyed under any reason the animal die within 3 days (Guyton and Hall, 2000). Now days the animals are subjected to different types of rations plus the increase of pollution due to modern life even in Villages the kidneys are subjected to different diseases, which are in excess as reported by many veterinarians working in the field. From the case history, increased gradual with fluctuating temperature, loos of appetite, poor body condition, all these sings which represented renal affection (Ahmed *et al.*, 2020). Moreover, the general clinical examination of the renal diseased

animals revealed signs of depression, hydration status and systemic abnormalities (Meyer *et al.*, 1992). The result of this study agreed to Bikaner *et al.*, 2016 in the cause of urinary tract infection are the bacteria and characterized by fever, colic and pyuria phenomenon and/or haematuria. PM finding in the present study revealed that the affected kidneys were enlarged and the capsule was easily stripped. The surfaces of which was mottled with circular red or sometimes yellow spots. Similar findings observed in kidneys of cattle suffered from pyelonephritis as a result of infection with *Corynebacterium renale*, *Arcanobacter pyogenes* and *Escherichia coli* (Braun *et al.*, 2008). The infection is more common in female than male this may be because female animals are predisposed to urinary tract infection because of their short urethra, urethral trauma, possibly hormonal effects and more reproductive system infection (Radostits *et al.*, 2007; Yeruham *et al.*, 2006).

The results indicated that 80% from all samples taken were positive bacteriologically for renal bacterial affections. Many organisms could be identified as *Staph. aureus*, *E. coli*, *Corynebacterium renal*, *Streptococcus spp.*, *Enterobacter faecalis* and *klebseilla pneumoniae*. Which agreed with (Normohammadzadeh *et al.*, 2003). The most common isolates observed were *Staphylococcus aureus* (25.0%) and *E. coli* (20.0%) (Table 2). Another study Mohammed *et al.* 2020 detected 46 isolates, divided to 44/56 (78.5%) *E. coli* and 2/4 (50%) *K. pneumoniae*. *E. coli* is a normal

inhabitant of lower intestine & is abundant in faeces and in the environment (Timonedey *et al.*, 1988 b). *E. coli* also, may be a common pathogen involved in urinary tract infection (Rebhum *et al.*, 1989). Serological characteristic of *E. coli* strains according to pathogenic mechanisms classified into four major categories enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC), which are represented by different serotypes based on O (cell-wall lipopolysaccharide), H (flagellar protein) and K (capsular polysaccharide or envelope) antigens (Collee *et al.*, 1996). In our study, 8 random *E. coli* serotypes were recorded to O111, O104, O26, O113, O91, O103 and O126.

Some of these isolates (O 26, O91, O113 and O 104) were also recorded by Cid *et al.* (2001) and Wani *et al.* (2004). The serogroup O78 which was isolated from hemorrhagic renal outbreak in sheep in India (Sharma *et al.*, 2003) and in Norway (Cecilie *et al.*, 2013) was not isolated in this study. Moreover, the serotypes O22, O55, O10 Bhat *et al.* (2008) which was not isolated in our study this may be attributed to different locality of each study. *Streptococcus spp.* and *Enterococcus faecalis* (7.5%) are also, opportunists & have been associated with pyelonephritis as recorded in sheep (Maxie and Prescott, 1993). *Pseudomonas aeruginosa* (5.0%) and *Klebsiella pneumoniae* (5.0%) were recovered in the present study and was considered as a least agent of renal affection in sheep as recorded also by (Timonedey *et al.*, 1988 c). Sarita *et al.* (2016).

One of the main causes of the emergence and transmission of resistant bacteria from food animals to people through the food chain is the unchecked use of antibiotics in livestock.

The most effective antibiotic against *E. coli* in the current study's antimicrobial sensitivity test was garamycin (30 g),

followed by kanamycin (Table 3). However, 100% resistance was detected when examine *E. coli* strains to tetracycline and erythromycin, 56.8% for penicillin but showed 100% sensitive to streptomycin and gentamycin, 90.9%; 75% sensitive to ciprofloxacin and trimethoprim respectively and 59% for chloramphenicol (Ahmed *et al.*, 2020). The results illustrated in Table 3 indicated sensitivity of *Staph aureus* strains to Garamycin 80% and Oxytetracycline 73%. These results come parallel with those recorded by (Fowler, 1998).

Regarding *Corynebacterium renale* the isolates were sensitive to Ampicillin 80% and Garamycin 76%. Contrarily, the data showed that 50% of Coryne were susceptible to chloramphenicol and that 100% of Coryne were sensitive to streptomycin, gentamycin, ciprofloxacin, and trimethoprim however, it was discovered that 100% of Coryne were tetracycline, erythromycin, and penicillin resistant. (Ahmed *et al.*, 2020). The highest sensitivity percent 90% in the current study was recorded on Streptococcus strains by Oxytetracycline followed by garamycin 60%. *Klebsiella pneumoniae* strains showed resistant for many antibiotics but sensitive to Garamycin 65% and Kanamycin 60%. In the genus *Klebsiella spp.*, *Klebsiella pneumoniae* is a species of aerobic Gram-negative bacterium that is the most significant human pathogen. It is the second-most significant microbe that causes urinary tract infections, behind *Escherichia coli* Finally, Antimicrobial sensitivity test revealed that garamycin (30 µg) and oxytetracycline (30µg) were the antibiotic of choice for combating bacterial renal infection. Silva/ *et al.* (2020) The result of PCR for amplification of internalin B (inlB) gene in *L. monocytogenes* showed that, in IB gene was amplified in all tested isolates, that disagreed with (Shen *et al.*, 2000).

CONCLUSION

From the present study, it was concluded that, *Staph. aureus*, *E.coli*. and *coryn.renal* are the most common pathogens which cause kidney problem in sheep. Recommendation for treating and controlling the clinical cases with the drug of choice based on the isolation of the causative organism and on the anti-biogram study which is garamycin and oxytetracycline. In PCR result assured that strains are listeria monocytogenes and have inlB virulence genes.

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دراسات على العدوى البكتيرية في الجهاز البولي للاغنام بمحافظة مطروح

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اشتمل البحث على ٥٠ حيوان من الاغنام من مناطق مختلفه في محافظة مطروح بالاضافه على بعض العينات من النسيج من الحيوانات المذبوحه من مختلف الاعمار والجنس اخذت عينات بطريقه معقمه من البول واستعمال الراسب المتكون من عمليه الطرد المركزي وايضا نسيج من الجهاز البولي للحيوانات المذبوحه لعمل الفحص البكتريولوجي.

عند فحص الجهاز البولي بعد ذبح الحيوان وجد تورم في بعض الكلى للحيوانات مع تنقيطها باللون الاحمر وعند اخذ قطع للنسيج وجد نزول دم او صديد في بعض الحيوانات. في الفحص البكتريولوجي تم عزل ٧ انواع مختلفه من البكتريا المسببه للمرض موجبه وسالبه الجرام وهي الايشريشيا كولاي (20.0 %) المكورات العنقودية الذهبية (٢٥%) ميكروب القيقح الازرق (٥,٠%) سلاسة الميكروب السبحى (٧,٥%) اينتيرو فيكالييز (٧,٥%) كورينباكتيريوم رينال (١٧,٥%) بالاضافه الى كليبيسيلا نيموني (٢,٥%) والستريا منوسيتوجين. (٢,٥%) الى جانب وجود ١٢,٥% من الحالات اظهرت اصابات مختلطة من اكثر من نوع من البكتريا . أظهر التنوع المصلى لعترات الميكروب القولونى عن وجود

O111, O104, O26, O113, O91 ,O103 and O126.

تم عمل اختبار حساسيه الميكروبات للمضادات الحيوية واطهرت النتائج ان الجاراميسين هو الدواء المختار لعلاج تلك الحالات فيما عدا الكورينبكتيريا رينال كان الاوكسييتتراسيكلين هو الاكثر فاعلية

اظهرت نتائج PCR أن سلالتين من عزلات الليستريا هما *Listeria monocytogenes* وبعضها يحتوي على جين InI .B