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Isolation and Identification of *Klebsiella pneumoniae* causing Urinary Tract Infection and Histopathological Changes of the Bladder and Kidney in Slaughtered Cattle

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Abstract

Aims:

Isolation and identification of *Klebsiella pneumoniae* from urine of breeders and cattle , relationship of infection rate with age and gender and histopathological changes in bladder and kidney of slaughtered cattle.

Methods: One hundred urine samples were collected from breeders and cattle raised in villages in Diyala province (50 from breeders, and 50 from cattle) from different ages, genders, and areas. The samples of urine were examined by microscopic examination ,cultured on MacConky and eosin methylene blue agar ,and subjected to full biochemical identification by vitek 2 system and histopathological study for bladder and kidney in slaughtered cattle.

Results:

Before collection of urine sample the main clinical signs showed in some affected cows with urinary tract infection (UTI) represented by weight loss and an abrupt reduction in feed intake and milk production specially dairy cow depression, weakness and frequent urine dribbling. Whenever in breeders represented clinically by cloudy urine, fever, strong unpleasant smell of urine, dark or bloody urine, flank or back pain, burning with urination. A total of 28/50 (56%) of urine specimens give positive urine culture among breeders, 4(8%) were *klebsiella pneumoniae*. A total of 44/50(88%) of urine specimens give positive urine culture among cattle,4(8%) were *klebsiella pneumoniae*. *Klebsiella Pneumoniae* appear pink mucoid colonies on macConky agar. Histopathological sections of kidneys shows granuloma formation (macrophages and lymphocytes) and damage in tubules, there is severe damage in glomeruli, tubules with hemorrhage.

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Urinary bladder shown thickening of mucosa with sloughing and infiltration of inflammatory cells with edema .

Keywords: Clinical, *Klebsiella pneumonia*, UTI, Histopathological changes, Slaughtered Cattle

Introduction

Urinary infection is abroad term of a variety of infectious diseases that affect the urinary system from a urethra to the kidneys [1].

Urine is the liquid waste that is expelled from the body through the urinary tract and urinary system in response to metabolic demands in humans and other animals. It includes important data about dietary consumption express to environmental contaminants, and human health [2].

UTIs in cattle can be caused by a variety of causes, including ascending infection from the urachus of new borne animals. An additional route for animal infection is through the vulva, which may be crucial as appoint of entry for some urinary tract infections. In cattle, *E. coli* as well as *Corynebacterium renale*, *Streptococcus spp*., *Proteus spp*. *Klebsiella spp*., and *Pseudomonas aeruginosa* are common causes of UTIs. In addition ,various conditions including bacteremia, septic catheterization, and post-calving diseases may put cattle at risk for UTIs. In addition to the elements linked to the intrinsic features of female anatomy, such the small urethra, elevated estrogen levels that might impact the urethras and the bladders epitheliums functional integrity, trauma and urinary tract infection after child birth. [3]. Pyelonephritis in cattle due to *Corynebacterium renale* used to be very common, the clinical disease has decreased markedly, with the majority of pyelonephritis cases in cattle now being due to *E.coli*, chronic cystitis were positive and *staphylococcus aureus*, *klebsiella* and *proteus mirabilis* were isolated [4].

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Current study aims to perform general examination of urine samples ,investigate effect of age and gender on infection rate of urinary tract infection ,isolation and molecular identification of *klebsiella pneumonia* from UTI and study the histopathological changes of bladder and kidney in slaughtered cattle .

Material and Methods

Samples

One hundred samples of urine were taken from cattle and breeders(50 from breeders, and 50 from cattle) from different ages, genders, and areas. Samples from cattle were taken by manual stimulation of perineum region. Breeders were asked to collected midstream urine. A few milliliters of urine were allowed to pass, then the sterile container was passed into the urine stream and removed before the avoid ends and transport immediately to the laboratory in iceboxes packet with ice .

Laboratory investigations:

The samples of urine were examined by microscopic examination ,cultured on MacConky and eosin methylene blue agar , and incubate for 24 hours at 37ºC, and store at 4ºC until use [5].The morphological characteristics included (colony size ,shape color elevation and texture) on culture media and biochemical tests. Gram staining was followed by Vitek 2 confirmation.[6],; [7].

Statistical Analysis

The data were analyzed using SPSS software, specifically version 23.00. P-values were computed. The chi-square test (X^2) was used to compare the data. A P value of less than 0.05 implies statistically significant results.

Results:

Source of urine samples

As shown in table (1) a total of 28/50 (56%) of urine specimens give positive urine culture among breeders, versus 22 out of 50 (44%) give negative urine culture. A total of 44/50 (88%) of urine specimens gives positive urine culture among cattle, versus 6 out of 50 (12%) give negative urine culture.

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Table (1): Source of urine samples

Source of urine samples	Number of bacterial isolates positive for urine culture	Total number (%)
Breeders	28(56%)	50(100%)
Cattle	44(88%)	50(100%)
* X^2 =12.698	*P value*= 0.00036	SD P<0.05

* X2= Chi-square , P = Probability value , SD= Significant difference

Relation between age of breeders with urinary tract infection due to *Klebsiella pneumoniae*

As shown in table (2), *Klebsiella Pneumoniae* was isolated from 4 out of 50, (8%) among breeders of the age groups(3-13),(14-24),(25-35),(36-46),(47-57),(58-68)and (69-79) years (No Significantly at P< 0.0.5) was reported between the age and infection with *Klebsiella pneumoniae*.

Table (2) : Relation between age of breeders with urinary tract infection due to Klebsiella pneumoniae and other bacteria (50 sample)

Total	Negative culture	Other bacteria	*Klebsiella pneumoniae*	Age(year)
3(6%)	2(4%)	1(2%)	0(0%)	3-13
7(14%)	3(6%)	3(6%)	1(2%)	14-24
18(36%)	7(14%)	10(20%)	1(2%)	25-35
10(20%)	7(14%)	3(6%)	0(0%)	36-46
6(12%)	2(4%)	3(6%)	1(2%)	47-57
5(10%)	2(4%)	2(4%)	1(2%)	58-68
1(2%)	1(2%)	0(0%)	0(0%)	69-79
50(100%)	24(48%)	22(44%)	4(8%)	Total
X^2 3.557	*P value*=0.614	NSD	P<0.05	

Relation between age of cattle with urinary tract infection due to *Klebsiella pneumoniae*

As shown in table (3), *K pneumoniae* was isolated from 4 out of 50, (8%) among cattle and calves . The age groups (3-9),(10-16),(17-23),(24-30),(31-37),(38-44),(45-51),(52-58) and (59-64) months Significantly,(P<0.05) was reported between the age and infection with *K pneumoniae* . The age of cattle was determined in months by asking breeders about their ages.

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Table (3) Relation between age of cattle with urinary tract infection due to *klebsiella pneumoniae*

Age (months)	*Klebsiella pneumoniae*	Other bacteria	Negative culture	Total
3-9	1(2%)	9(18%)	0(0%)	10(20%)
10-16	0(0%)	5(10%)	0(0%)	5(10%)
24-30	1(2%)	10(20%)	0(0%)	11(22%)
31-37	1(2%)	6(12%)	2(4%)	9(18%)
38-44	0(0%)	0(0%)	0(0%)	0(0%)
45-51	1(2%)	6(12%)	2(4%)	9(18%)
52-58	0(0%)	0(0%)	1(2%)	1(2%)
59-64	0(0%)	4(8%)	1(2%)	5(10%)
Total	4(8%)	40(80%)	6(12%)	50 (100%)
X^2 = **10.227** **2.381** ***P value*= 0.036** **SD P<0.05**				

Relation between gender of breeders with urinary tract infection due to *Klebsiella pneumoniae*

As shown in table (4), the positive urine culture results showed (28) (11 male and 17 female) from (50) samples of breeders, The isolated bacteria in male is(10) *E coli* , and (1) *Klebsiella pneumoniae*. The isolated bacteria in female is (12) *E coli*, and (3) *Klebsiella pneumoniae* and(1)*Enterobacter aerogenes*,(1) *Enterobactercloaca complex* (No Significantly P<0.05) was reported between the gender and infection with *K pneumoniae* .

Table (4) shows the numbers and types of bacteria isolated from 50 urine sample of breeders (male and female).

Bacteria	Male NP / NE	Female NP / NE	Total NP / NE
Klebsiella pneumoniae	1/ 11	3/ 17	4/ 28
E.coli	10/11	12/17	22/ 28
***Enterobacter cloacae* complex**	0/11	1/17	1/28
Enterobacter aerogens	0/11	1/17	1/28
Total	11/11	17/17	28/ 50
X^2 = **1.786** ***P value*= 0.181** **NSD P<0.05**			

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Relation between gender of cattle with urinary tract infection due to *Klebsiella pneumoniae*

As shown in table (5), The positive bacterial culture results were(44) (10 male and 34 female) isolates from a (50) urine samples of cattle. The isolated bacteria in male is (8) *E.coli*, and (1) *Klebsiella pneumoniae* and (1) *pseudomonas aeroginosa*. The isolated bacteria in female were(29) *E.coli*, and (3) *Klebsiella pneumoniae*,(2) *Enterobactercloaca complex* (Significantly at P<0.05) was reported between the gender and infection with *K. pneumoniae* **.**

Table (5) shows the numbers and types of bacteria isolated from 50 urine sample of cattle (male and female).

Bacteria	Male NP / NE	Female NP / NE	Total NP / NE
Klebsiella pneumoniae	1/ 10	3/34	4 /44
E. coli	8/10	29/34	37/44
Enterobacter cloacae complex	0/10	2/34	2/44
pseudomonas aeruginosa	1/10	0/34	1/44
Total	10/10*	34/34*	44/50
X^2 = 23.377	***P value*= 0.0000013**	****SD P<0.05**	

NP= number of positive / NE= Number of positive culture examined

Histopathological examination of kidneys and urinary bladders of Cattles

The figure (1) shows normal kidney in cattle, figure (2) histopathological section of kidney with granuloma formation (macrophages and lymphocytes) and damage in tubules. There is severe damage in glomeruli , tubules with hemorrhage figure (3). Urinary bladder shown thickening of mucosa with sloughing figure (4),and infiltration of inflammatory cells with edema figure (5).

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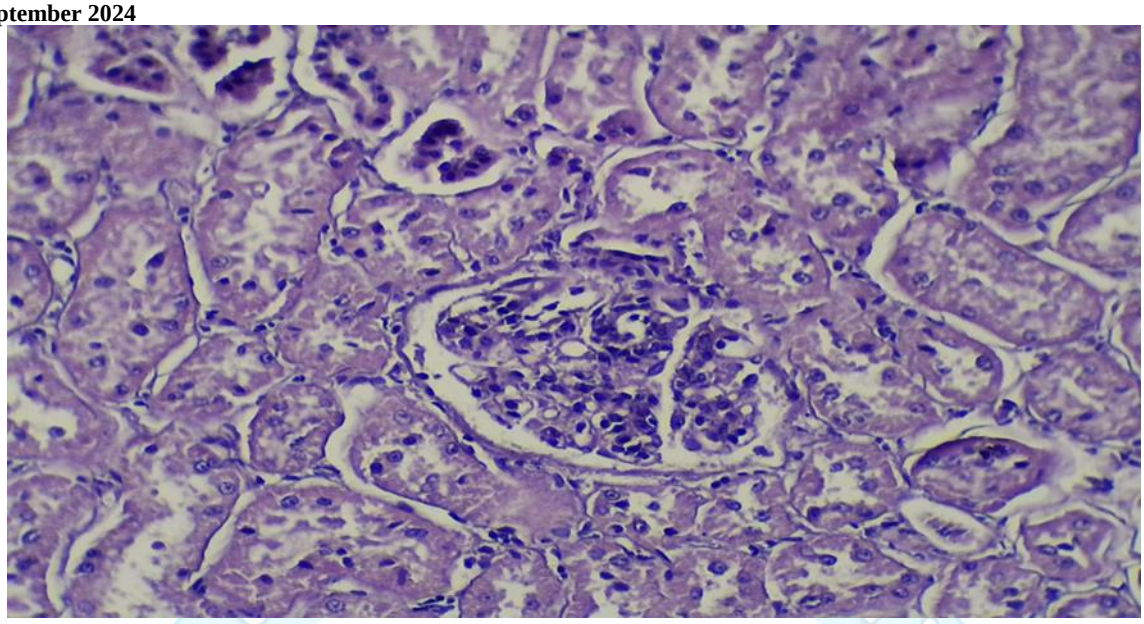

Figure (1):paraffin embedded (sections 5µm), histological section showed : normal kidney in cattle. (H&E) stain X 20.

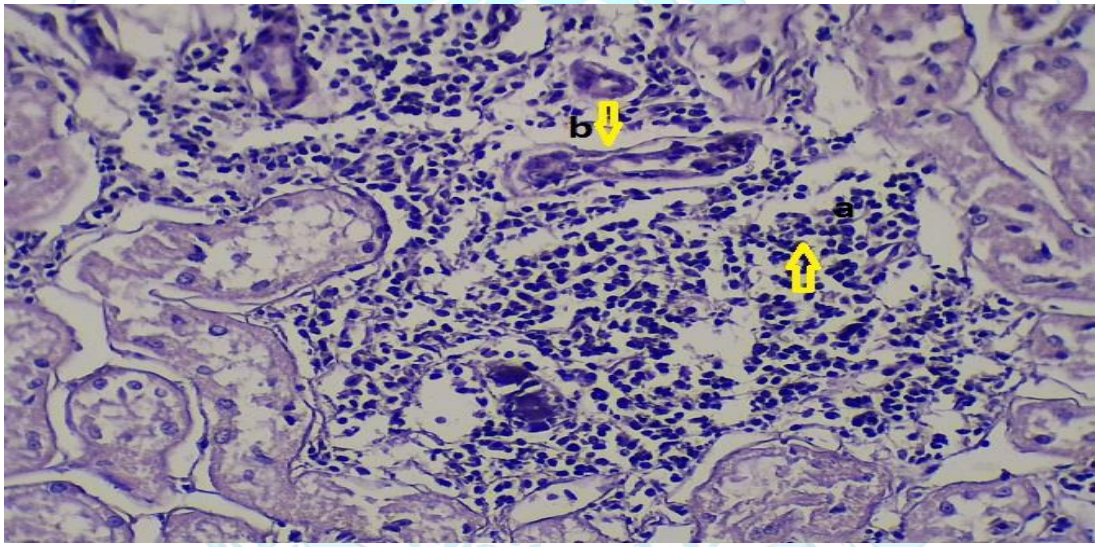

Figure (2): kidney in cattle paraffin embedded (sections 5µm), histopathological section showed : a) granuloma formation (macrophages and lymphocytes) b) damage in tubules . (H&E) stain X 20.

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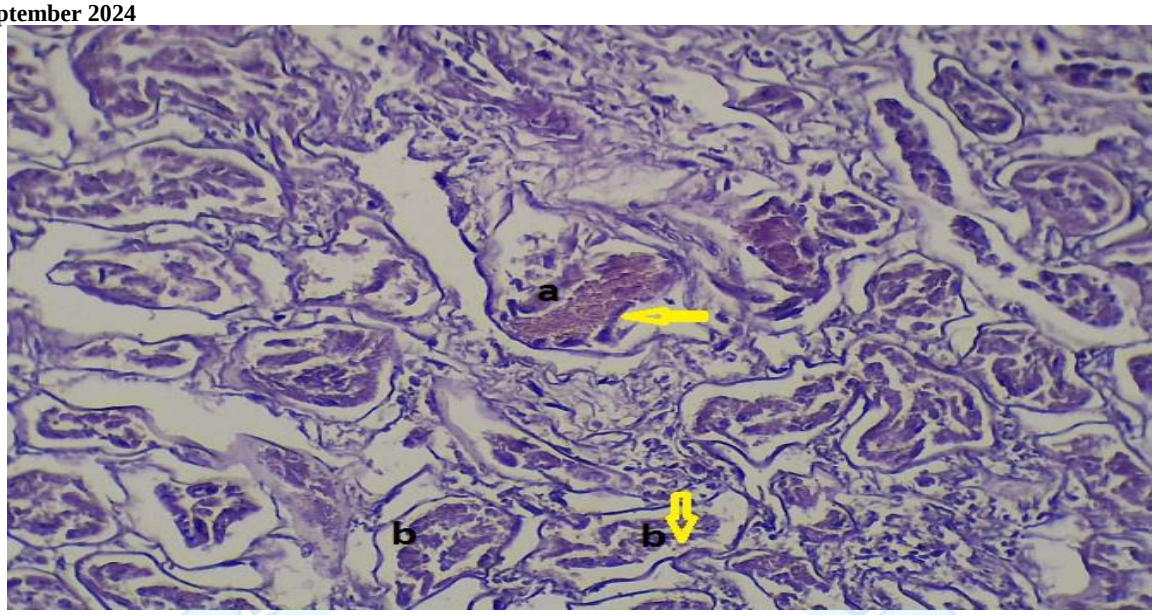

Figure (3): kidney in cattle paraffin embedded (sections 5µm), histopathological section showed: a) hemorrhage b) damage in tubules. (H&E) stain X 20.

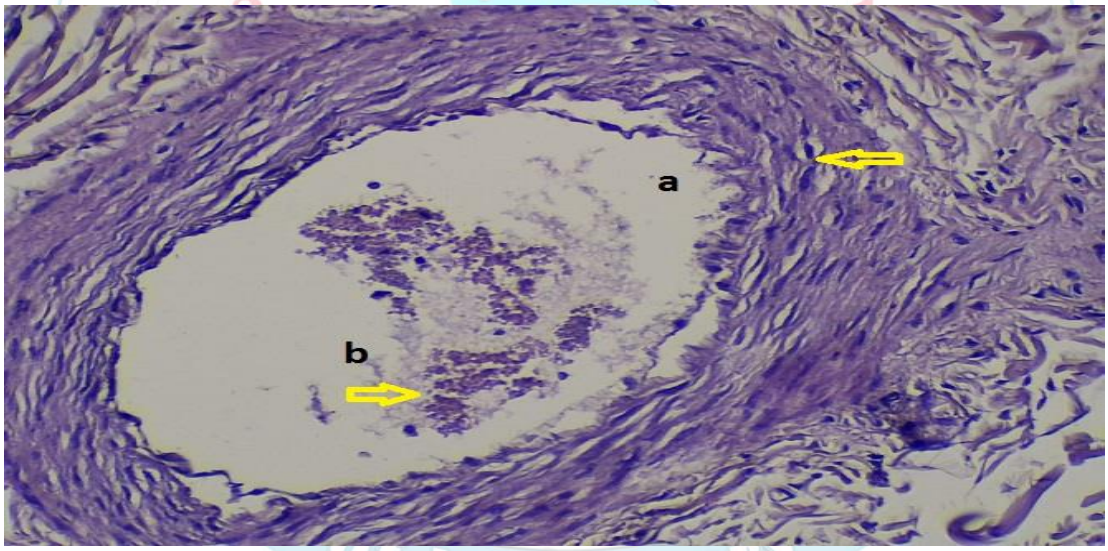

Figure (4) : Bladder in cattle paraffin embedded sections (5µm), histopathological section showed: a) thickening of mucosa b) sloughing . (H&E stain) X 20.

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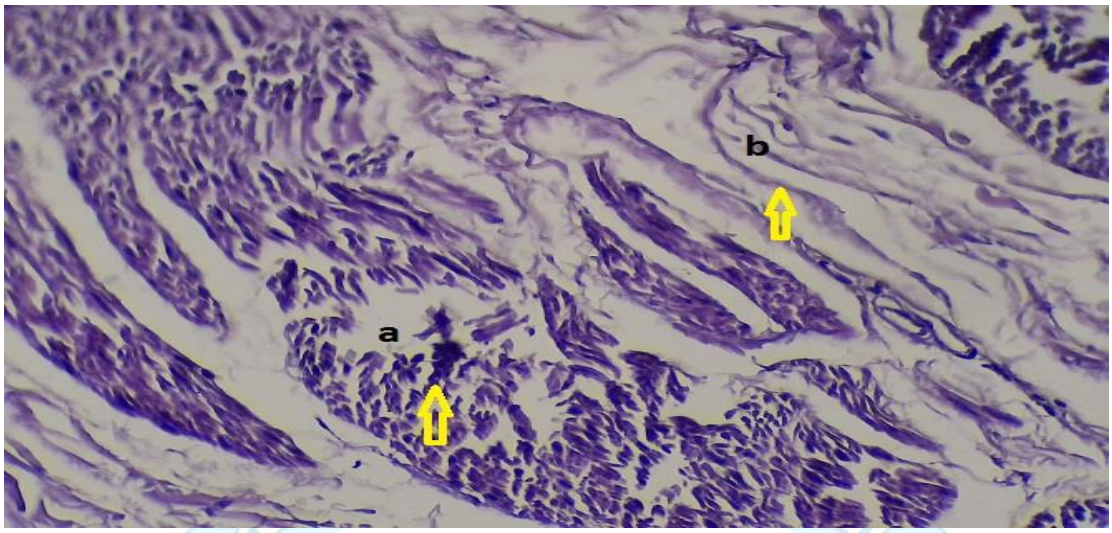

Figure(5): Bladder in cattle paraffin embedded sections (5µm), histopathological section showed: a) infiltration of inflammatory cells b) edema . (H&E stain) X 20.

Disscusion

The findings of the current study showed a total of 28/50 (56%) of urine specimens give positive urine culture among breeders, versus 22 out of 50 (44%) give negative urine culture, this agree with [8] in India who found UTI to be (53.82%) , and [9] in republic of trenadad to be (49%). While our study disagree with [10] and [11]study, which showed (4.2%) and (10.86) % respectively of positive culture.

In this study the results of age of breeders infected with *K pneumoniae* was isolated from 4 out of 50, (8%) among breeders of the age groups (14-24),(25-35),(47-57) and (58-68) years, this agree with [12] and [13]. This is because most people at this age are married and more susceptible to UTI infection , especially in females, because they are of pregnancy age, and disagree with [14]and [15] which indicated the incidence is higher within the age group (41 and above) .

Klebsiella pneumoniae is widely speeded in cattle that mean came play arole in the zoonotic transmission to breeders through direct contact with infected animals, contaminated equipment, and unpasteurized milk

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derivatives.The results of age of cattle infected with *K pneumoniae* was isolated from 4 out of 50, (8%) among cattle. The age groups (3-9),(24-30),(31-37) and (45-51) months, this result agree with [16] who found UTI infection may occur in calves between 7 days to three months of age . In order to show that bacteria are transmitted from animals to humans through contaminated equipment and hands

The results of this study show that the infection rate in females higher than males in breeders .The infection rate among females was(6%) while male infection rate (2%) and this result agree with [14],[17],[13],but disagree with [18] who found that uropathogenic *K pneumoniae* in males (31.6%) was more dominant than females (7.3%) and found that the females were more prone to contracting *E coli* infection , whereas infection with *Enterobacter* species and *klebsiella pneumoniae* were more common in males .

In cattle the infection rate in females higher than males.The infection rate among females was(6%) while male infection rate (2%).Our results agree with[19],but disagree with [6];[20], who stated that the prevalence on infection in male was more than in female.

Histopathologically our finding come agree with [21]and [22] who found that the specimens showed focal and diffuse inflammatory cells infiltration in calves kidneys, in addition to a degenerative cells inside the renal tubules and inflammatory cells infiltrating around the kidneys glomeruli after *Klebsiella pneumoniae* isolation and experimental infection in mice kidneys[21];[22]. There was severe damage in glomeruli, tubules with hemorrhage which agree with [23] who found that renal hemorrhage, tubular necrosis and glomerular and tubular atrophy in the kidneys of cattle slaughtered at two animal slaughterhouse as a result of infection . In the current study a section of urinary bladder shown thickening of mucosa with sloughing this result come agree with [24] who found that infected urinary bladder of sheep, with hyperplasia of epithelium, infiltration of inflammatory cells

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and thickening of sub mucosa. In current study the result shows infiltration of inflammatory cells with edema, this datum agree with [25] who found in the study on cattle and buffalo that the lamina propria was intensely edematous and neutrophil cell was infiltrated, hemorrhage and hyperemia found in addition to congestion inside the blood vessels and prevascular cellular infiltration , indicating uretritis and cystitis.

Conclusion

From results we can concluded that the *Klebsiella pneumoniae* is the most common bacteria among the causative organism of UTI which cause high infection in cows and females and may be a risk factor for the development of urinary disorders.

References

[1] Al Lawati H,. Blair B,M., and L, Jeffrey. Urinary Tract Infections: Core Curriculum. American Journal of Kidney Diseases. 2023; 83(1):90-100. doi.org/10.1053/j.ajkd.2023.08.009.

[2] Zha, Y., and Huang, S. Urine Biochemistry and Inspection[M]. Beijing, China: People's Medical Publishing House, 2019; 5–815. DOI:10.3389/frans.2021.812301.

[3] John AS, Mboto CI, Agbo B. A review on the prevalence and predisposing factors responsible for urinary tract infection among adults. Euro J Exp Biol. 2016; 6(4):7-11. https://www.researchgate.net/publication/303651684.

[4] Nikvand, A.A., Haji Hajikolaei, M.R., Ghadrdanmashhadi, A.R., Ghorbanpour, M., Mohammadian, B. Bacteriological study of urine and its relationship with histopathological findings of bladder and kidney in river buffalo (*Bubalus bubalis*). Iranian Journal of VeterinaryMedicine,2014;8(3):157-161. https://journals.ut.ac.ir/article_51886_6b70a015a430f49818e92407b0199b0a.pdf

[5] Quinn, P., Carter, M., Markey, B. & Carter, G. ClinicalVeterinary Microbi-ology, Mosby Elsevier Limited. Edinburgh London, New York. Oxford, Philadelphia, St Louis, Sydney, Toronto; 2004.

[6] Fatihu, M.Y.,and Addo, P.B. Isolation of Corynebacterium renale from slaughtered cattle at the Zaria abattoir in Nigeria. Rev Elev Med Vet Pays Trop 1991;44(2):160–161. https://pubmed.ncbi.nlm.nih.gov/1818361.

[7] Feng, P., S.D. Weagant, and K. Jinneman. Bacteriological Analytical Manual Diarrheagenic Escherichia

coli. 2016;USFDA, 8th Edition, Chapter 4. pp:1-27. https://pubmed.ncbi.nlm.nih.gov/18183 61.

[8] Prakash, D. and Saxena, R. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut city, India. ISRN Microbiology, 2013; 13. DOI: 10.1155/2013/749629.

[9] Orrett. F. A. Urinary tract infections in general practice in a rural community in South Trinidad. Saudi Medical Journal, 2001;vol. 22, no. 6, pp. 537‑540. https://pubmed.ncbi.nlm.nih.gov/1 1426248.

[10] Bigwan E. I. and Elijah. D. Prevalence of *Escherichia coli* among uropathogens in asymptomatic bacteriuria in a Nigerian Tertiary School in Jos, Nigeria, International Journal of Biomedical and Advance Research, 2013;vol. 4, no. 3, pp. 198‑202. DOI:10.7439/ijbar.v4i3.250.

[11] Kothari, A. and Sagar, V. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: a multicenter study. J Infect Developing Countries , 2008;2(5):354-358. DOI: 10.3855/jidc.196.

[12] Sarhan, A. & Tawfiq, M. T. A survey on Urinary Tract Infections in Baghdad. Al-Ma'moon Col. J 2013; 22, 235-245. https://www.researchgate.net/publi cation/343837594_A_survey_on_ Urinary_Tract_Infections_in_Bag hdad.

[13] Derdera, E. M.,Sarrab, A. R.,Dughaej, J. A.,Almakki ,T.M.and Rahoma , N.Z. Urinary tract infection caused by Klebsiella bacteria for patients attending the Zintan General Hospital and the DialysisHospital. 2020;https://www.researchgate.net /publication/339726519.DOI:10.1 3140/RG.2.2.20768.33287.

[14] Bulati, K. A Study on drug of choice for the UTI patients in al-khoms, Libya. Journal of Dental and Medical Sciences, 2016;10(15): 60-63. https://www.iosrjournals.org/iosr-jdms/papers/Vol15-Issue%2010/Version-6/M1510066063.

[15] Garba, Ali. M., K. and Abdallah, M. Antibiotic susceptibility profile of bacteria responsible for urinary tract infection (UTI). South Asian Journal of Biological Research,2018; 1(1): 12-27. http://aiipub.com/wp-content/uploads/2018/10/SAJBR-181002-030114_fp.pdf.

[16] Yeruham, I., Elad, D., Avidar, Y., Goshen, T., Asis, E.(). Four year survey of urinary tract infections in calves in Israel. Veterinary Record, 2004; 154,

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P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

204–206. DOI: 10.1136/vr.154.7.204.

[17] Agila, A., Almahdi, A., Mohammed, H., Saleh, Z., Safe, S., Abdesalem, A. and Ismail, F. Urinary tract infection in eastern Libya during one decade and preventive healthy diet, The 2nd Libya Conference of Medical Laboratory,2018;15-18. https://www.researchgate.net/publication/324533577.

[18] Varughese L. R. and Beniwal V.High quinolone resistance pattern among enterit pathogen isolated from patients with urinary tract infection**,** Indian Journal of Biotechnology,2015;14:167-171. https://www.researchgate.net/publication/282328682.

[19] Yeruham, I., Elad, D., Avidar, Y., and Goshen, T. A herd level analysis of urinary tract infection in dairy cattle. Vet. J.2006;171,172–176. DOI: 10.1016/j.tvjl.2004.04.005.

[20] Haji Hajikolaei, M.R., Normohammadzadeh, F., Zahraei S.T., Tabatabaei, A.M. Clinical and paraclinical study on the prevalence of pyelonephritis in cattle slaughtered at Tehran province abattoirs. J Fac Vet Med Univ Tehran, 2002;56:123–123. https://www.sid.ir/paper/35238/en.

[21] Ismail, H. K. Histological Lesions Of Slaughtered Calves Kidnys In Mosul SlaughterHouse.Bas.J.Vet.Res.2015;Vol.14,No.1. https://www.researchgate.net/publication/328733743.

[22] Abadullah S. M. , Zghair Z.R. Isolation of *Klebsiella pneumoniae* from urine of human and cattle in Baghdad city with histopathological study experimentally in mice. Int.J.Adv.Res.Biol.Sci.2016;3(10): 3845.DOI:http://dx.doi.org/10.22192/ijarbs.2016.03. 10.006.

[23] Ajayi, O. L., Antia, R. E., Awoyomi, O.J., Oladipo ,T. M. & Adebowale ,O.O. Cultural, histochemical, and immunohistochemical detection of pathogenic *Leptospira* species in the kidneys of cattle slaughtered in two abattoirs in Southwest Nigeria. Journal Of Immunoassay And Immunochemistry, 2020;Volume 41, Issue 3, 337-353. https://doi.org/10.1080/15321819.2020.1736096.

[24] Mohammed, Y. J., Mustafa J. Y. and Abdullah A. R. Isolation and Molecular Study of Some Bacterial Urinary Tract Infections of Sheep in Basrah Province. Iraqi Journal of Agricultural Sciences, 2020; 51(3):885-893. DOI: https://doi.org/10.36103/ijas.v51i3.1043

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University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

[25] EL-Naser, E. M.A., EL-Nisr, N. A.,. Hassan, A. M., Khamis, G.F., Aamer, A.A., and Yosif, N. M.A. Bacteriological, Pathological and Biochemical Studies on The Urinary Tract Affections on Cattle and Buffaloes. Assiut Vet. Med. J. 2011; Vol. 57 No. 130. DOI: 10.21608/AVMJ.2011.176862.