Urinary tract bacterial infection of local Iraqi buffaloes (*Bubalus Bubalis*) in Mosul city

O. M. Al-Iraqi[1], A. Y. Shareef[2] and S. H. Dhahir[3]
[1,3]Department of Veterinary Internal and Preventive Medicine, College of Veterinary Medicine, [2]Department of Biology, College of Science, [1,2]Mosul University, [3] Baghdad University, Iraq.
E-mail: tebavet@yahoo.com
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Summary

This study is conducted on the samples of urine obtained from buffaloes. Eighty six urine samples were obtained from local buffaloes of different ages, of both sex in Mosul abattoir. The results showed that urine of infected animals was cloudy, dark yellow to reddish color with putrid odor of low specific gravity. Microscopic examination of urine sediment demonstrated increased number of pus cell, erythrocytes, epithelial cell, presence of casts and crystals/ high power filed. Chemical examination revealed elevated urine pH and protein content. Serum examination displayed increase levels of urea and creatinine compered to normal urine samples. Culture of urine samples confirmed 24 positive samples (27.90%). *Corynebacterium renale* being the most isolated organisms 12(50%), *Trueperella pyogens* 5(20.83%), *Staphylococcus aureus* 4(16.66%) and 1(4.16%) for each of *Acinetobacter lwoffii*, *Corynebacterium pilosum* and *Corynebacterium cystitidis*. Male buffalo calves at age 1-3 years revealed high percentage (18.60%) of urinary tract infection, while low percentage of urinary tract infection existed by female buffaloes age more than 3 years (6.98%).

Keywords: Buffaloes, Urine, Bacteria.

Introduction

Urinary tract infection (UTI) in bovine often results from ascending infection with *Corynebacterium renale* or *Escherichia coli*. Other less causative organisms include various coliform species and other members of the *C. renale* group. Renal infection through hematogenous route is less common and may result from bacteremia with *Salmonella* species, *Actinomyces pyogenes* (1 and 2). In buffalo calves urinary tract infection include pyelonephritis and cystitis caused by *E.coli*, *C. renale*, *Staphylococcus aureus*, *Streptococcus pyogenes* are not uncommon and are mostly suspected clinically and confirmed on post slaughtering inspection (3). *E. coli*, *Staphylococci* and *Pseudomonas* are the most common pathogens which cause kidney problem in buffaloes (4). UTI is much more common in female ruminant than in males because of relatively short urethral length in females and urinary tract contamination and trauma during parturition (1). There are many researches reporting a urinary tract infection in cattle (3 and 5-10) and in buffaloes (4 and 11). Although UTI in cattle was studied in Iraq (2, 12 and 13), there is no research about UTI in Iraqi buffaloes, this study was conducted to identify the common bacterial infection of urinary tract infection in Iraqi buffaloes (*Bubalus Bubalis*) in Mosul city.

Materials and Methods

Eighty six urinary bladder samples were obtained from apparently healthy slaughtered buffalo aged 1-8 years of both sexes (34 male and 52 female) at the period from April to July 2013 from Mosul abattoir. Samples were taken under aseptic condition and transmitted directly for laboratory examinations.

Urine samples examinations: Urine samples which collected from urinary bladder by using sterile syringes under aseptic aspiration technique, which subject to physical and chemical examination using commercial urine strips (England Co.) and examined microscopically according to (14).

Serum examination: Blood Urea Nitrogen (BUN), after letting the specimens and reagents at room temperature, working reagent (R1+R2) I mL were mixed with 5 µL of specimen then waited for 4 minutes, then base (R3) were added and waited 8 minutes and read absorbance at 600 nm against blank and calculated the result according to Biolabo (Biolabo Co, France).

Serum Creatinine (Ct): Working reagent (R1+R2) I mL were mixed well with 100 µL

of specimen, after 30 seconds recorded absorbance A1 at 490 nm against reagent blank. Exactly 2 minutes after the first read, recorded absorbance A2 and calculated the result according to Biolabo (Biolabo Co, France).

A loopful of urine sample was cultured on blood agar and MacConCkeys' agar, incubated aerobically at 37°C for 48 hrs. The suspected colonies were examined morphologically, the biochemical tests were done according to (14-17), some bacterial colonies were identified using Vitek 2 Technology. The VITEK ® 2 gram positive and gram negative identification cards are intended for use with VITEK® 2 system for automated identification of most gram positive and gram negative organisms. They are based on established 42 biochemical tests measuring carbon source utilization, enzymatic activities and resistance (bio Merieux, USA). Data where analaysed statisticaly for means and significant with T-test by use SPSS program (Microsoft, Ver. 11.5, USA).

Results and Discussion

The physical characteristics of urine were determined in 86 tested slaughtered buffaloes, of these 34 urine samples showed abnormality in color (dark yellow to reddish), transparency (cloudy and turbid), foam (> 10 second) and specific gravity (1.010±0.003) compared to 52 normal samples (Table, 1).

Table, 1: Physical examination of urine samples of slaughtered buffaloes.

	Physical characteristic N (86)	Normal N (52)	Diseased N (34)	
			Negative N (10)	Positive N (24)
1	Color	Yellow	Dark yellow, reddish	
2	Transparent	Clear	Turbid	
3	Foam	<10 seconds	>10 seconds	
4	Specific gravity (Mean ±SD)	1.023 ±0.002	1.010 ±0.003*	

*** Significant difference at (P<0.05).**

Gross urine examination revealed a cloudy, turbid and milky, brownish sometimes reddish with putrid odor urine. This suggests an inflammation associated with pyelonephritis, cystitis or possible vulvovaginitis. Clinically affected animals revealed different changes in urine according to the disease stage, it appeared turbid in early stage then becoming fitfully blood tinged, and in the final stage it was almost firmly blood stained. Milky and dark color in most urine samples result from presence of epithelial cells, pus cells, damaged tissue and salt crystals lead to loss of transparency of urine. These results are compatible with those of (13, 18 and 19).

The lower urine specific gravity readings in the present study were in agreement with results of (20-22) which indicated renal disease with tubular or interstitial damage, the persistently diluted (hypotonic) urine in an azotemic or dehydrated animals is usually indicative of dysfunction of tubules, The concentrating ability of healthy animal kidney will produce urine SG between 1.020 and 1.050 (23 and 24). Refractometry can be used reliably in routine laboratory practice for most urine samples for detection specific gravity (25).

The biochemical urine tests in normal control and culture negative and positive are summarized in (Table, 2). There were significant difference (P<0.01) between positive urine samples and both of normal and negative samples in pH in which positive sample had the highest pH level (8.9±0.3), while there were no difference between normal and negative urine samples in pH. Urine protein showed significant difference (P<0.01) between the three groups and the highest protein level were in the positive urine samples (160±36.55). Glucose, bilirubin, ketone and nitrate were negative in all samples.

Table, 2: Biochemical urine tests of normal control, negative and positive bacterial urine culture samples of slaughtered buffaloes (Mean ±SD).

Biochemical urine tests	Normal control N (52)	Negative N (10)	Positive N (24)
Urine pH	7.2 ± 0.3^{b}	7.9 ± 0.2^{b}	8.9 ± 0.3^{a}
Protein mg/Dl	89.5 ± 12^{c}	92.50 ± 19.65^{b}	160 ± 36.55^{a}
Glucose	0	0	0
Bilirubin	0	0	0
Ketone	0	0	0
Nitrate	0	0	0

Results, indicate different changes in urine contents, included increase in pH level (8.9±0.3) in positive urine samples, which

occurred due to increase in activity of some bacteria during infection like *Corynebacterium renale* which possesses the enzyme urease that allows metabolism of urea and production of ammonia changing urine pH to alkalinity. Same results were also found by (26 and 27). Mild elevated in pH and proteinuria level negative samples compared with normal animals and positive samples might be due to early disease process or as compensatory mechanisms of renal parenchyma or after treatment of diseased animals, *C. renale* increases urine pH more than 7.9 which facilitates adherence to the urinary system (21 and 27). Out of 17 cattle infected with pyelonephritis, 3 cows displayed decrease urine pH and 5 cows revealed increase urine pH (21). Noticeable proteinuria agreed with (3 and 12) could be due to glomerular diseases, while milder proteinuria could be associated with tubular lesions (28).

The microscopic urine examination in normal control, culture positive and negative are summarized in (Table, 3).

Table, 3: Microscopic examination of urine in control and negative and positive bacterial urine culture samples of slaughtered buffaloes (Mean±SD).

Microscopic urine test	Normal N (52)	Negative N (10)	Positive N (24)
Pus cell/ HPF	4±1^{c}	8±1^{b}	18±2^{a}
Erythrocytes /HPF	3.3±1^{c}	14.60±5.70^{b}	27.22±3.94^{a}
Epithelial cell /HPF	5.89±0.95^{c}	7.87±0.7^{b}	12.87±2.16^{a}
Crystals/HPF	0	0	10±2**
Casts/HPF	0	0	10±2**

**** Significance differences at (P<0.01).**

The positive urine samples showed elevated values in all tested parameters compared to normal control urine samples. There were significant difference (P<0.01) between urine positive and urine normal control samples in pus cells, erythrocytes and epithelial cells, where as the crystals and casts showed significant difference (P<0.05). Ten urine samples negative for bacterial culture showed elevated urine pus cells, erythrocytes and epithelial cells. Pus cells, erythrocytes, epithelial cells, crystals and casts were increased in positive urine samples (18±2, 27.22±3.94, 12.87±2.16, 10±2, 10±2)/ High power field respectively. These are in accordance with (6 and 18). Traditional epithelial cells might be seen in inflammation, trauma and neoplasms. Hematuria and pyuria appear mostly in pyelonephritis due to inflammatory lesions of the ureters and bladder. In acute cystitis urine examination revealed blood, pus and desquamated epithelial cell with a strong ammonia odor, moreover in less severe cases the urine might be only turbid where as in chronic cases it might be without abnormality on gross inspection (24). Desquamated epithelial cells, erythrocytes and leukocytes revealed on microscopic examination of urine sediment may ascribe to highly vascular papillary projections which may be eroded causing the urine to be blood stained or contain large clots of blood. Presence of pus and blood in urine may suggest cystitis or embolic nephritis as well as pyelonephritis. Cystitis occurred due to the introduction of infection into the bladder when trauma occurred as in late pregnancy or when there is stagnation of the urine. Casts are organized tubular structures that vary in appearance depending on their composition. They occur only when the kidney is involved in the disease process (28 and 29). Increase in RBC, pus cells and epithelial cells in negative urine samples for bacterial culture might be due to infection of urinary system with virus or fungus, or as a result of post treatment with antibiotics or tumors (25 and 30).

The BUN and Ct measurements in normal control and culture negative and positive are summarized in (Table, 4). The positive urine samples showed elevated values in BUN and Ct compared to normal control urine samples. There were significant difference (P<0.05) between urine positive and urine normal control samples in BUN and Ct, where as 10 urine samples negative for bacterial culture showed high normal level of BUN and Ct.

Table, 4: Detection of blood urea nitrogen and serum creatinine in normal control and negative and positive bacterial urine culture samples of slaughtered buffaloes (Mean ±SD).

Serum BUN and Ct	Normal N (52)	Negative N (10)	Positive N (24)
Blood urea nitrogen mg/dL	18±2^{c}	27±4^{b}	37.5±3^{a}
Creatinine mg/dL	1±0.2^{c}	1.7±0.7^{b}	3±0.5^{a}

Blood urea nitrogen and serum creatinine were examined in the serum, BUN was within normal ranges (18±2) in normal control animals samples, although some of samples show high level within the normal range (27±4). Positive samples showed abnormal high values of urea (37.5±3). Creatinine was within the normal levels (1±0.2) in normal control, while the positive samples showed abnormal high values, these results agree with those of (21 and 27). Some of the positive bacterial urine samples had normal serum creatinine and blood urea nitrogen. Urea and creatinine concentration in serum do not increase considerably more than normal level until about 60-75% of nephrons are damaged. Cattle occasionally have disproportionate increase in serum Ct compared to BUN, and this could be due to an increase in noncreatinin chromogens that falsely increase serum Ct but do not affect BUN (24 and 31).

Isolated bacterial species were been identified as follows out of total 86 urine samples, 24 samples (27.90%) were positive and 62 samples (72.09%) were negative. Out of 24 bacterial isolates 12(50%) was *C. renale*, 5(20.83%) *T. pyogenes*, 4(16.66%) S. *aureus*, and one isolate (4.16%) of each of *A. lwoffii*, *C. pilosum*, *C. cystitidis* (Table, 5).

Table, 5: Number and percentage of isolated pathogens from urine of slaughtered buffaloes.

	Isolated Bacteria	Number of isolates	Percentage %
1	*Corynebacterium renale*	12	50
2	*Trueperella pyogens*	5	20.83
3	*Staphylococcus aureus*	4	16.66
4	*Acinetobacter lwoffii*	1	4.16
5	*Corynebacterium pilosum*	1	4.16
6	*Corynebacterium cystitidis*	1	4.16
	Total positive	24	27.91
	No growth	62	72.09
	Total	86	

Corynebacterium renale was the predominant bacteria isolated from UTI. These results agreed with (3, 19 and 32), this might be attributed to that infection was accompanied by production of urease that metabolizes urea leading to the high affinity of this bacteria to urinary system. *C. renale*, *C. pilosum* and *C. cystitidis* (previously named *Corynebacterium renale* type I, II and III respectively) cause pyelonephritis and cystitis in cattle (33). The results of urine bacterial culture were in agreement with those of other reports on cattle pyelonephritis and cystitis (5, 21 and 27). Corynebacterium group are the most isolated organism in buffaloes due to their ability to survive for long period in soil especially humus where buffaloes live which attached to the vulval epithelial cells and results in retrograde infection leading to pyelonephritis, also it is well attached to the well-differentiated epithelial cells in urine and render the urine pH to alkaline (29). It is reported that *Corynebacterium renale* and *Corynebacterium cystitidis* survived in soil for long periods (8-9 weeks respectively), while *Corynebacterium pilosum* survived for more period (at lest 30 weeks). The incidence is greater when there is a high level of nitrogen in the diet leading to high excretion of urea (34).

Corynebacterium renale is the most common cause of cystitis, urethritis and pyelonephritis. *Corynebacterium pilosum* is a milder pathogen often resulting in an uncomplicated cystitis. The virulence of these species is attributed to pili, which mediate adhesion to epithelial cells of the bladder and urease enzyme, which is responsible for hydrolyzing urea and releasing nitrogen, and renalin (mainly in *Corynebacterium renale*) that may play a role in lysis of host cells (5). *Trueperella pyogenes* isolated approximately from 20.83% of affected cases with cystitis and pyelonephritis, this result are more than the percentages of other researches (13 and 21), that's may be due to bad management of buffaloes fields. It is usually found on mucous membranes, adhesion to host tissue is facilated by neuraminidases and extracellular matrix-binding proteins and infection arises when it gains entrance to deeper tissue as a result of various injuries predisposing to ascending pyelonephritis as pregnancy or parturition, trauma to the vagina or urethral mucosa, or obstruction of the lower urinary tract or other infections including those caused by viruses, mycoplasma and other bacteria (5 and 35). It produces a range of virulence factors, the most significant of which is a hemolytic exotoxin and pyolysin, these toxins are cytolytic for neutrophils and macrophages. (36). *Trueperella pyogenes* (previously named

Archanobacterium pyogenes), It causes pyelonephritis or embolic purulent nephritis in cattle (33),

Staphylococcus aureus was isolated from 4 urine samples (16.66%) and agreed with (4 and 11). It has many potential virulence factors and has especial concern in human and veterinary medicine through antimicrobial resistant strain (14). *Acinetobacter lwoffii* was isolated from one urine sample (4.16%). This is in accordance with results of (13). *Acinetobacter* spp. Glucose-non-fermenting, gram negative bacteria. It is of minor veterinary importance. Prevalently presents in water, soil, food, milk, and sewage. It is considered as apart of normal flora in animals and humans. It can cause nosocomial infections, especially in immune-compromised patient (14). Of the total tested buffaloes, the infected males at age less than one year were 2 calves (2.33%) where as of 1-3 years showed 16 infected calves (18.60%). In females at 1-3 years there was one infected (1.16%) and at age of 3-15 years include 5 infected buffaloes (5.81%) (Table, 6).

Table, 6: Age and Number of tested slaughtered buffaloes.

		Age					
		≤1 year		1-3 years		3-15 years	
Sex	N.	-ve	+ve	-ve	+ve	-ve	+ve
Male	52	30	2 2.33%	4	16 18.60%	0	0
Female	34	0	0	8	1 1.16%	20	5 5.81%
Total	86	30 34.88%	2 2.33%	12 13.95%	17 19.76%	20 17.2%	5 5.81%

The results reveal high percentage (18.60%) of UTI in male buffalo calves at age 1-3 years, Similar results were recorded by (3 and 11). This may be due to fattening programs at this age of male buffalo calves, include feeding of high concentrate feed with overcrowding, contact, contamination and ammonia liberation predispose to block the ureters by obstructive urolithiasis, inflammatory swelling or debris lead to urine stasis and establishment of bacterial growth in the urinary bladder and ascending infection of urinary system occur (29 and 37).

The higher percentage of infection in female buffaloes (5.81%) at age of 3-15 years compared with 1-3 years, similar results were reported by (9 and 38) this result could be attributed to pregnancy and parturition at age of more than 3 years, at this age predisposing factors could damage the urinary tract (5 and 39).

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الخمج البكتيري للجهاز البولي في الجاموس العراقي المحلي (*Bubalus bubalis*)

أسامة موفق عبد الجبار[1] و أديبة يونس شريف[2] و سالم حمد ظاهر[3]

[3،1] فرع الطب الباطني، كلية الطب البيطري، [2] قسم علوم الحياة، كلية العلوم، [2،1] جامعة الموصل، [3]جامعة بغداد، العراق.

E-mail: tebavet@yahoo.com

الخلاصة

أجريت هذه الدراسة للتحري عن أخماج الجهاز البولي في الجاموس المحلي بعزل المسببات الجرثومية والتغيرات الفيزيائية والكيميائية لعينات البول. شملت الدراسة فحص 86 عينه من الجهاز البولي حُصِلَ عليها من الجاموس المحلي بمختلف الأعمار ومن الذكور والإناث والمذبوح في مجزرة الموصل. أجري الزرع الجرثومي وتسجيل التغيرات الفيزيائية والكيميائية لعينات البول, حيث اظهرت عينات البول للحيوانات المصابة وجود عكارة ولون ابيض داكن الى محمر مع رائحة فاسدة وقلة في الكثافة النوعية، في حين تبين من الفحص المجهري لراسب البول وجود زيادة في الخلايا القيحية وكريات الدم الحمر والخلايا الطلائية والقوالب والبلورات، أما الفحوصات الكيميائية للبول فقد كشفت عن زيادة في الأس الهيدروجيني والبروتين، في حين وجد من فحص المصل ازدياد في قيم اليوريا والكرياتينين مقارنة بالعينات المأخوذة من الحيوانات السليمة. أكد الزرع الجرثومي لعينات البول وجود 24 عينة موجبة (27.90%)، وسجلت أعلى نسبة من العزلات لجراثيم *Corynebacterium renale* 12 (50%) تلتها جراثيم *Trueperella pyogenes* 5 (20.83%) وجراثيم *Staphylococcus aureus* 4 (16.66%) وكانت اقل العزلات 1 (4.16%) لكل من *Acinetobacter lwoffii* و *Corynebacterium pilosum* و *Corynebacterium cystitidis* . أظهرت ذكور عجول الجاموس بعمر 1-3 سنه اعلى نسبة خمج للجهاز البولي (18.60%)، في حين أقل نسبة خمج للجهاز البولي ظهرت في أناث الجاموس بعمر أكثر من 3 سنوات (6.98%) .

الكلمات المفتاحية: جاموس، بول، جراثيم.