Bacteriological study of urine and its relationship with histopathological findings of bladder and kidney in river buffalo (*Bubalus bubalis*)

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Key words:
buffalo, bacteriology, bladder, kidney, pathology

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Received: 14 May 2014
Accepted: 9 July 2014

Abstract:

BACKGROUND: Cystitis and pyelonephnitis in ruminants most often result from ascending urinary tract infection (UTI). **OBJECTIVES:** Investigating the prevalence of urine infection and its relationship with histopathological findings of bladder and kidney. **METHODS:** This study was carried out on 353 slaughtered buffalos (143 female and 210 male) at Ahvaz abattoir, southwest of Iran. After slaughter, urinary samples were taken directly from bladder by sterile syringe. The bladder and kidney were inspected and samples from them were taken for histophatological study. All of the urine samples were incubated for isolation of bacteria on blood agar and MacConkey. Biochemical characterizations of the isolates were performed according to Bergey's manual of systemic bacteriology. Histopathological samples were taken from representative parts of each bladder and kidney and were stained with haematoxylin and eosin. **RESULTS:** Out of the 353 urine samples, 19 (5.38%) were positive. The percentages of infected cattle in female and male were 6.3% and 4.8%, respectively. These bacteria were isolated: *E.coli* (21%), *Staphylococcus* spp. (31.6%), *Streptococcus* spp. (15.8%), *Proteus* spp. (15.8%), *Klebciella* spp. (5.3%), *Yercinia* spp. (5.3%), and *Actinimyces* and *Pasteurelle* spp. (5.3%). In histopathological examination, 51(14.4%) and 5 (1.4%) of the buffalos had chronic cystitis and pyelonephritis, respectively. Bacteria were isolated only in 3 cases of chronic cystitis and 2 cases of pyelonephritis. Statistical analysis showed there was no relationship between isolation of bacteria from urine with cystitis and pyelonephritis. **CONCLUSIONS:** Some cases of chronic cystitis are not able to isolate of bacteria from urine and for causing pyelonephritis they need predisposing factors which caused urine retention.

Introduction

Cystitis, uretritis, and pyelonephnitis in ruminants most often result from ascending urinary tract infection (UTI) with *Corynebacterium renale* or *Escherichia coli*. Less common causative organism includes *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Arcanobacterium pyogenes*, *Corynebacterium pseudotuberculosis*, and other members of *C.renale* groups. Renal infection via the hematogenous route (suppurative embolic nephritis) is much less common but may result from bacteriemia with such agents as *Salmonella* species, *Arcanobacterium pyogenes* or in small ruminants, *Corynebacterium pseudotuberculosis*

(Radostits et al., 2007; Smith, 2009; Yeruham et al., 2006). These organisms have been isolated from both apparently healthy cattle and they show signs of urinary tract infection, because they need predisposing factors for pathogenesis such as urolithiasis, difficult pregnancy, late pregnancy, prostatic hypertrophy, bladder paralysis, and ureteral anomalies (Normohammadzadeh et al., 2003; Radostits et al., 2007; Smith, 2009; Yeruham et al., 2006).

Interstitial nephritis is rarely recognized as a cause of clinical disease in farm animals although it is a frequent postmortem finding in some species. Focal chronic interstitial nephritis, also called "white spotted kidney" is a common finding in clinically healthy cattle at slaughtered and has frequently been assumed to be related to current of prior infection with leptospira spp. However, studies of "white spotted kidney" in cattle at abattoir indicate that neither leptospira spp. nor active infection by other bacteria are associated with the lesion. Unfortunately, a definitive diagnosis of leptospirosis is difficult to make. Most diagnostic laboratories do not attempt to isolate leptospires because of their fragile nature, cost, complexity of the isolation media, and prolonged incubation period. Therefore, recognition of leptospiral infection has been generally based on serological evidence, as very few isolations have been reported from naturally infected animals (Radostits et al., 2007).

There is a lack of knowledge in urinary tract pathological lesions in cattle than other species. This difference between species may be due to the low incidence of clinical nephrological diseases in this animal. However, most urinary bladder and renal lesions are subclinical and they might have remarkably higher frequencies than expected; such lesions could result in the poor production of the involved animals (Yeruham et al., 2006; Divers et al., 1982). Studies were carried out to investigate the prevalence of bacterial infection of urine and its relationship with cystitis and pyelonephritis in Iran (Normohammadzadeh et al., 2003; Haji Hajikolaei et al.); however, there are no documents of same study which showed the prevalence of UTI in buffalo. Therefore, this study was carried out on slaughtered buffalos from Ahvaz, Iran.

Materials and Methods

This study was carried out on 353 slaughtered buffalos (143 female and 210 male) at Ahvaz abattoir, south west of Iran, from March 2012 to February 2013. Before slaughtering, the age of animals was documented according to dental formulas and divided to two groups (≤2.5 and >2.5 years). After slaughter, urine samples were taken directly from bladder by sterile syringe. All of the urine samples were incubated for isolation of bacteria on blood agar and MacConkey and the plates were incubated at 37^{o}C for 24-48h. After this time, plates were examined for characteristic colonies. Smears from the growths were stained by gram technique for identification of cellular morphologies. Biochemical characterizations of the isolates were performed according to Bergey's manual of systemic bacteriology (Holf et al., 1993).

The samples of kidneys and bladders were taken for histopathological study. One to three blocks of tissue with 4 to 5mm thickness were taken from representative parts of each bladder and kidney and were placed in 10 percent buffered formalin. These were embedded in paraffin wax and sections 5µm thick and were stained routinely with haematoxylin and eosin.

The data were analyzed statistically by using Chi-square and Fisher's - exact test with significance level at 0.05.

Results

Out of the 353 urine samples, 19(5.38%) were positive and the following organisms were isolated, *Escherichia coli* (21%), *Staphylococcus* spp. (31.6%), *Streptococcus* spp. (15.8%), *Proteus* spp. (15.8%) and *Klebciella* spp. (5.3%), *Yercinia* spp. (5.3%) and in one sample mixed infection *Actinomyces* and *Pasteurella* spp. (5.3%) (Table 2).

The percentage of infected buffalo in females and males were 6.3% and 4.8%, respectively, and there was no significant difference between these groups ($p>0.05$) (Table 1).

The percentage of infected buffalo in ≤2.5 and >2.5 years old were 5.2% and 5.5%, respectively, and there was no significant difference between these age groups ($p>0.05$) (Table 1).

Table 1. Age and sex distribution of bacterial infection in examined buffalo.

Sex	Female		Male		Total	
Age(years)	Positive	Negative	Positive	Negative	Positive	Negative
≤2.5	1(2.6%)	38(97.4%)	8(6.0%)	126(94.0%)	9(5.2%)	164(94.8%)
>2.5	8(7.7%)	96(92.8%)	2(2.6%)	74(97.4%)	10(5.5%)	170(94.5%)
Total	9(6.3%)	134(93.7%)	10(4.8%)	200(95.2%)	19(5.3%)	334(94.7%)

Table 2. Type of bacteria isolated from urine of examined buffalo.

	female		male		Total	
	≤2.5	>2.5	≤2.5	>2.5	≤2.5	>2.5
	(year)					
Escherichia coli	0	0	3	0	3	0
Staphylococcus aureus	0	1	0	0	0	1
Staphylococcus epidermidis	0	2	1	0	1	2
staphylococcus equorum	1	1	0	0	1	1
Streptococcus equinus	0	1	0	0	0	1
Streptococcus bovis	0	1	0	0	0	1
Streptococcus suis	0	2	1	0	1	2
proteus mirabilis	0	0	1	0	1	0
klebsiella	0	0	0	1	0	1
yersinia enterocolitica	0	0	1	0	1	0
actinomyces viscosus+pasteurella aerogenes	0	0	1	0	1	0
Total	1	8	8	2	9	10

Table 3. Correlation between chronic cystitis and pyelonephritis with bacteria isolated from urine of examined buffalo.

Histopathology	Chronic cystitis		Pyelonephriris	
Urine culture	Positive	Negative	Positive	Negative
Positive	3	16	2	17
Negative	48	286	3	331

In histopathological examination of bladder there were pathological findings related to chronic cystitis (Figure 1) in 50(14.4%) of buffalo. Pyelonephritis (Figure 2) was seen in 5(1.4%) of buffalo.

Urine culture of three cases of chronic cystitis were positive and *Staphylococcus aureus*, *klebsiell*,a and *proteus mirabilis* were isolated and the other cases no bacteria was isolated.

Urine culture of three cases of pyelonephritis were positive and *staphylococcus equorum* was isolated from one case and the another there was mixed infection *actinomyces viscosus* and *pasteurella aerogenes*, but in the three others of pyelonephritis no bacteria was isolated (Table 3).

Discussion

In the present study, 5.3% bacterial culture of the urine samples collected from the slaughtered buffalos were positive and *E.coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *staphylococcus equorum*, *Streptococcus equinus*, *Streptococcus bovis*, *Streptococcus suis*, *proteus mirabilis*, *klebsiella*, *yersinia enterocolitica*, *actinomyces viscosus*, and *pasteurella aerogenes*, were isolated. In the study of Fatihu and Add (1991), Normohammadzadeh et a1. (2003), Haji Hajikolaei et al. (2012), respectively 12.5%, 10.2%, and 12.98% of urine samples from slaughtered cattle were positive and *C.renale*, *C. pilosum*, *E.coli*, *Staphylococcus* spp., *Streptococcus* spp. and *Arcanobacterium pyogenes* were isolated (Fatihu and Add, 1991; Normohammadzadeh et a1., 2003; Haji Hajikolaei, et al., 2002).

In this study, similar to the study of Normohammadzadeh et al. (2003) there was no significant difference between bacterial infection of urine in female and male; however, this difference in the study of Fatihu and Addo (1991) was statistically significant and prevalence on infection in male was more than female. Although 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). In histopathological examination of bladder, there were pathological findings related to chronic cystitis in 50(14.4%) and pyelonephritis in 5(1.4%) of buffalo. The prevalence of chronic cystitis (14.4%) in this study is higher than that of Hernando et al.'s survey (5.3%) in slaughtered cattle in Toronto abattoir (Hernando et al., 1990). In Hernando et al.'s survey average age of the animals examined was slightly less than 2.5 years; however, more than half of the buffalos were more than 2.5 years old. Somvanishi et al. (2012) reported 50% of urinary bladder of 94 cattle and 142 buffaloes collected from Bareilly, Uttar Pradesh, India, showed acute (18.64%), and chronic (31.35%) cystitis.

Urine culture in three cases of chronic cystitis were positive and *staphylococcus aureus*, *klebsiella* and *proteus mirabilis* were isolated, but two cases of pyelonephritis were positive and *staphylococcus equorum* was isolated from one case and the other one

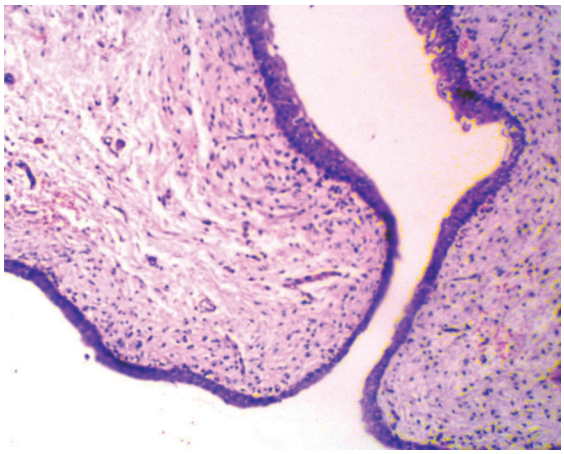

Figure 1. Chronic cystitis, increased lamina properia of submucosal layer of urinary bladder, included proliferation of collagen fibers &fibrocytes (H&E, 10x).

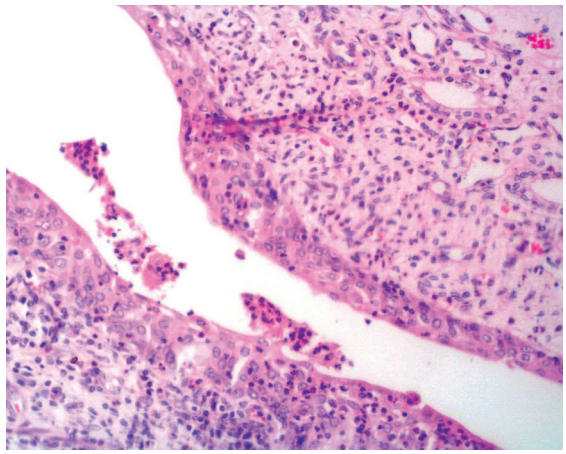

Figure 2. Chronic pyelonephritis with pus mass included neutrophils in renal pelvis of kidney (H&E, 20x).

had mixed infection of *Actinomyces viscosus* and *pasteurella aerogenes*. Pathogenic organism such as *E.coli*, *Arcanobacter pyogenes*, *Corynebacterium renale*, *Streptococcus* spp., *Staphylococcus* spp., *Psedumonas* spp., *Bacillus* spp., and *Alcaligenes* faecalis were isolated from acute and chronic cystitis (Herenda et al., 1990).

In the survey of Ibrahim et al. (2008) on slaughtered buffalos, pathological studies revealed that 70% of rejected kidney showed the histo-pathological picture of pyelonephritis and seven different bacteria of both gram positive and gram negative such as *E.coli*, *Staphylococcus aureus*, *Psedumonas aeroginosa*, *Streptococcus* spp., *Enrococcus faecalis*, *Corynebacterium* spp. and Klebseilla pneumonia were recovered from affected kidney.

C.renale, *Streptococcus* spp., *Psedumonas aeroginosa*, *Proteus* spp., *Klebseilla* spp. and Oligella urethralis were isolated from 17 calves and 19 cows that had signed were the main characteristics of urinary tract infection (Yeruham et al., 2008).

In the study of Rosenbaum et al. (2005), twenty-one rejected kidneys (0.87 per cent) had gross signs of pyelonephritis. In all of the kidneys, the findings were consistent with a chronic rather than an acute infection. One species of bacteria was cultured from 12 of the kidneys, and two species of bacteria were cultured from six. The most commonly isolated bacteria were *E. coli*, *Arcanobacterium pyogenes*, *Corynebacterium renale*. The other bacteria cultured were *Corynebacterium cystitidis*, *Corynebacterium* species, *Streptococcus* species group G and *Enterococcus faecalis*. *E. coli* was cultured from all the kidneys from which two species were isolated. No bacteria were cultured from two of the kidneys and no significant bacteria were cultured from the other one (Rosenbaum et al., 2005). *E. coli*, *Arcanobacterium pyogenes*, *Corynebacterium renale* were isolated in urine of 14 from 17 cows with pyelonephritis (Braun et al., 2008).

In this study, *Corynebacterium renal* group were not isolated from the examined buffalos. Although pyelonephritis in cattle due to *C.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*. The reason for the decrease in *C.renale* isolation from clinical cases is unclear; however, it is probably related to changes in diet concentrates with an associated decrease in urine pH; other potential reasons could be the widespread use of beta-lactam antibiotics and the marked decrease in urethral catheterization in order to obtain a urine sample in cows suspected to be ketotic (Radostits et al., 2007).

One of the urinary tract defense mechanism against bacterial infection and colonization is shedding of mature epithelial cells with attached bacteria through normal micturition. Factors involved in ascending UTI include the dose and virulence of the bacterial challenge, the presence of urogenital trauma or abnormal vulvar conformation, obstetric manipulation, bladder catheterization, and urine retention (Smith, 2009). All of the bacteria

isolated from urine are causative organisms of urinary tract infection specially pyelonephritis, embolic nephritis, and cystitis; however, only in three cases of chronic cystitis two cases of pyelonephritis urine culture were positive and in 48 cases of chronic cystitis and 3 cases of pyelonephritis we could not isolate bacteria. On the other hand, in 14 cases that urine culture was positive there were not any histopathological findings related to urinary tract infection. Therefore, there was no relationship between occurrence of urinary infection and histopathological changes of kidney and urinary bladder. It is emphasized that bacterial agent of urinary tract infection isolated from apparently healthy cattle and for pathogenesis their need predisposing factors which caused urine retention and in some cases of chronic cystitis we not to able isolate bacteria.

Acknowledgments

The authors would like to acknowledge the research vice chancellors of Shahid Chamran University for financial support of this research. We would appreciate the executives and workers of Ahvaz abattoir and the technicians of large animal internal medicine section, pathology and microbiology laboratories of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz.

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مجله طب دامی ایران، ۱۳۹۳، دوره ۸، شماره ۳، ۱۶۱-۱۵۷

مطالعه باکتریولوژی ادرار و ارتباط آن با یافته‌های هیستوپاتولوژی مثانه و کلیه در گاومیش رودخانه‌ای

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(دریافت مقاله: ۲۴ اردیبهشت ماه ۱۳۹۳، پذیرش نهایی: ۱۸ تیر ماه ۱۳۹۳)

چکیده

زمینه مطالعه: التهاب مثانه، التهاب حالب و پیلونفریت در نشخوارکنندگان، اغلب ناشی از عفونت‌های بالارونده مجاری ادرار می‌باشد. **هدف:** بررسی شیوع آلودگی باکتریایی ادرار و ارتباط آن با ضایعات هیستوپاتولوژی مثانه و کلیه در گاومیش. **روش کار:** ۳۵۳ رأس گاومیش (۱۴۳ رأس ماده، ۲۱۰ رأس نر) ارجاعی به کشتارگاه اهواز تحت مطالعه قرار گرفتند. بعد از کشتار نمونه ادرار از مثانه اخذ گردید. نمونه‌های ادرار برروی محیط‌های مک‌کانکی و بلاد آگارکشت و بعد از ۴۸-۲۴ ساعت انکوبه از نظر رشد باکتری بررسی شدند. باکتری‌های جداشده با استفاده از روش‌های بیوشیمیایی تعیین هویت شدند. از بافت کلیه و مثانه نمونه برای بررسی‌های هیستوپاتولوژی تهیه گردید. نمونه‌ها با استفاده از رنگ‌آمیزی هماتوکسیلین و ائوزین رنگ‌آمیزی شدند. **نتایج:** ازمجموع ۳۵۳ نمونه ادرار (۵/۳۸٪) ۱۹ نمونه دارای باکتری بودند که این مقدار در گاومیش‌های ماده و نر به ترتیب ۶/۳٪ و ۴/۸٪ بوده است. (۲۱٪) *E.coli*، استافیلوکوکوس (۳۱/۶٪)، استرپتوکوکوس (۱۵/۸٪)، پروتئوس (۱۵/۸٪) و کلبسیلا (۵/۳٪)، یرسینیا (۵/۳٪)، اکتینومایسس و پاستورلا ۵/۳٪ همزمان جدا شدند. در بررسی‌های هیستوپاتولوژی (۱۴/۴٪) ۵۱ و (۱/۴٪) ۵ نمونه به ترتیب واجد دارای التهاب مزمن مثانه و پیلونفریت بودند. از ادرار ۳ رأس مبتلا به التهاب مزمن مثانه و ۲ رأس مبتلا به پیلونفریت باکتری جداشد. بررسی‌های آماری نشان داد ارتباطی بین آلودگی ادرار با التهاب مزمن مثانه و پیلونفریت وجود ندارد. **نتیجه‌گیری نهایی:** نتایج حاصل از این مطالعه نشان می‌دهد که در تمام موارد ضایعات عفونی دستگاه ادراری امکان جدا نمودن باکتری وجود ندارد و در دام‌های فاقد ضایعه دستگاه ادرای نیز باکتری‌های مسبب بیماری حضور دارد و برای ایجاد ضایعه نیاز به عوامل مستعد کننده دارند.

واژه‌های کلیدی: گاومیش، کلیه، مثانه، باکتریولوژی، پاتولوژی

*)نویسنده مسئول: تلفن: ۳۳۶۰۰۷۸ (۶۱۱) ۹۸+ نمابر: ۳۳۳۲۰۴۰ (۶۱۱) ۹۸+ *Email: mhajih@scu.ac.ir*