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Occurrence of bacteremia, bacteriuria and bacteriuria-related bacteremia in dogs and cats with chronic kidney disease. A pilot study

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ABSTRACT

In human medicine, major infections are the most significant and critical non-cardiovascular complications in patients affected by chronic kidney disease (CKD), with bacteriuria being the primary source of bloodstream infections and its evolution toward sepsis. The availability of data on prevalence of bacteremia and its association with bacteriuria in dogs and cats with CKD is limited. The aim of this observational cross-sectional study was to determine the occurrence of bacteremia, bacteriuria, and bacteriuria-related bacteremia in dogs and cats affected by CKD. Client-owned dogs and cats with a documented history of CKD undergoing disease follow-up were enrolled. Each included animal underwent a comprehensive physical examination, clinico-pathological and microbiological analyses of blood and urine, along with molecular detection of the 16S rRNA bacterial gene in blood. Aseptically collected blood and urine were obtained through jugular venipuncture and cystocentesis, respectively. After collection, blood and urine samples underwent bacteriological culture within one hour. In the population enrolled, 2/47 dogs and 1/41 cats presented bacteriemia. Moreover, 8/47 dogs and 6/41 cats presented a positive urine culture. Additionally, in one out of the 47 dogs, the same pathogen was identified from blood and urine samples, with a final diagnosis of urosepsis. No instances of bacteriuria-related bacteriemia were observed in the cat population. In conclusion, this study shows a low prevalence of bacteremia and confirms a high prevalence of bacteriuria in companion animals affected by CKD. Moreover, a low prevalence of bacteriuria-related bacteremia was also found.

1. Introduction

Chronic kidney disease (CKD) is the most recognized form of kidney disease as well as an important cause of morbidity and mortality in dogs and cats (O'Neill et al., 2013; Marino et al., 2014). It is defined as an irreversible process characterized by persistent (≥3 months) loss of function or structural changes in the kidneys, most often leading to a progressive decline in kidney function and to the onset of typical extra renal complications (Polzin, 2011).

In human medicine, emerging evidence indicates that infections represent the most substantial and critical non-cardiovascular complications in patients affected by CKD (Ishigami and Matsushita, 2019), with urinary tract infections (UTIs) being the most represented (Xu et al., 2017). Additionally, patients with varying extents of CKD, ranging from albuminuria even in the presence of preserved renal function, to mild, moderate, or severe renal failure, face an increased risk of bacteremia and sepsis due to community-acquired bloodstream infections when compared to non-CKD patients (Rojas et al., 2013; Dagasso et al., 2020). UTIs are the primary source of bacteremia and potential progression to urosepsis among human CKD patients (Rojas et al., 2013; Dagasso et al., 2020).

Several pathways potentially link CKD to infections, including chronic inflammation, uremic toxins retention, and metabolic disorders. Compromised immune function, as evidenced by observed lymphocyte-related abnormalities in cellular and humoral immunity, decreased neutrophil function, and oxidative stress, contributes to increased susceptibility to infections in human beings affected by CKD (Ishigami and Matsushita, 2019).

Consistent with human observations, canine studies revealed altered neutrophil oxidative metabolism, increased oxidative stress, and cell apoptosis among dogs with uremia (Bosco et al., 2017). This process contributes to an increased susceptibility to infections in uremic patients

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(Bosco et al., 2017). Additionally, feline CKD is associated with oxidative stress, which is characterized by reduced plasma antioxidant capacity and increased neutrophil oxidative metabolism (Keegan and Webb, 2010).

However, unlike in humans, there is a lack of data on the prevalence of infections, including bloodstream infections, in dogs and cats with CKD, with the only available data concerning the elevated prevalence of bacteriuria and UTIs (Mayer-Roenne et al., 2007; White et al., 2013; Foster et al., 2018; Dorsch et al., 2019; Lamoureux et al., 2019; Uva et al., 2022).

Therefore, this study aims to determine the occurrence of bacteriemia and bacteriuria in dogs and cats diagnosed with CKD stages 1 to 4 according to the International Renal Interest Society (IRIS) staging system (IRIS staging guidelines of CKD, 2023) by clinico-pathological and bacteriological analyses of blood and urine, including molecular detection of the 16S rRNA bacterial gene in blood. In addition, the occurrence of bacteriuria-related bacteremia was also investigated when a positive blood culture was obtained.

2. Materials and methods

2.1. Study population and data collection

From January 2021 to June 2023, client-owned dogs and cats, of any age and breed, both male and female in any reproductive state, were recruited if affected by a stable and at least 3-month-long CKD classified as IRIS stage 1 to 4. In this regard, CKD diagnosis had to be based on the presence of: (i) "inappropriately dilute" urine (IRIS staging guidelines of CKD, 2023) without another identifiable cause of polyuria, namely due to renal disease, and/or (ii) increased serum creatinine concentrations without identifiable cause of pre-renal or post-renal azotemia, and/or (iii) proteinuria of renal origin confirmed by a urine protein to creatinine ratio (UPC) $>$ 0.2 in cats and $>$ 0.5 in dogs and/or dipstick measurements of 3+ positive (500 mg/dL) in cats and 2+ positive (100 mg/dL) or above in dogs with inactive urine sediment. After diagnosis, CKD was staged according to the IRIS guidelines (IRIS staging guidelines of CKD, 2023).

For each included animal, signalment data (i.e., breed, age, and sex), baseline information (e.g., clinical history, concomitant treatments relevant to CKD, previous laboratory findings), and clinical signs reported by the owner suggestive of UTIs (i.e., gross hematuria, stranguria, pollakiuria and periuria) were recorded (Wood, 2017). Furthermore, a complete physical examination, including body condition score (BCS), muscle condition score (MCS) and systolic blood pressure evaluation, was performed. Systemic inflammatory response syndrome (SIRS) was recognized when at least 2 out of 4 and 3 out of 4 clinical indicators, known as the SIRS criteria, were met in dogs and cats, respectively. The SIRS criteria included abnormal body temperature (fever/hypothermia), abnormal heart rate (tachycardia/bradycardia), tachypnoea, and white blood cell abnormalities (leukocytosis or leukopenia, bandemia) (Spillane et al., 2023). Renal imaging was performed to identify any anatomical abnormalities or structural changes.

Animals with known or suspected to be affected by acute kidney injury within the previous 28 days, azotemia of pre-renal or post-renal origin, congenital and acquired anatomical defects of the genitourinary tract, urolithiasis, pyometra, infectious prostatic disease, treated with antimicrobials or immunosuppressive or immunostimulant drugs within the previous 4 weeks, undergoing urethral catheterization or previously urethrostomized, as well as animals known or suspected to be affected by comorbidities interfering with the immune system (i.e. hyperthyroidism, diabetes mellitus, hyperadrenocorticism, neoplasia, retrovirus infections) were excluded.

2.2. Sample collection

Peripheral blood samples were collected by jugular venipuncture for haematological and biochemical analyses. A 2 ml blood sample was collected in a BD Vacutainer™ K3-EDTA tube for CBC measurement. Serum samples were obtained by centrifuging 5 ml of blood collected in a BD Vacutainer® clot activator serum tube at 1500 x g for 15 mins. Blood samples for hemoculture (at least 2.5 ml) were aseptically collected in BD Vacutainer Plus Citrate Plasma Tubes (Becton, Dickinson and Company, US) by jugular vein puncture after aseptic skin preparation. All urine specimens were sampled by ultrasound-guided cystocentesis and stored in BD Vacutainer® urinalysis preservative tubes (5 ml) for physical-chemical examination and BD Vacutainer® Plus C&S Boric Acid Sodium Borate tubes (2 ml) for microbiological analysis.

After collection, blood, serum, and urine samples were promptly refrigerated until being sent to the reference laboratory. The samples were processed within 24 h. Urinary and blood samples collected for bacteriological culture were immediately sent to the microbiology laboratory and processed within 1 h of collection.

2.3. Laboratory tests

Results from CBC (Siemens, ADVIA 2120), serum biochemistry (Beckman Coulter, Clinical Chemistry Analyzer AU680) and electrophoresis (SEBIA, Capillarys 2 Flex Piercing) were achieved with the same methods in all tested samples. Additionally, microscopic blood smear examination was performed for all samples. In the context of urinalysis, USG was measured using a refractometer (Leica Vet 360, Misco Products Division, Cleveland, OH, USA), and urine dipstick examination was interpreted according to the manufacturer's recommendations (Combur 9 Test, Roche, Rotkreuz, Switzerland). Based on USG, urines were classified as diluted (USG $<$1.013), moderately concentrated (USG 1.013 to 1.029 [dog] or 1.034 [cat]) or concentrated (USG $>$1.030 [dog] or $>$1.035 [cat]) (IRIS staging guidelines of CKD, 2023). To determine the UPC ratio, the protein concentration (mg/dl) was assessed using the pyrogallol red-molybdate assay, while the serum creatinine (mg/dl) was measured through the Jaffé method in undiluted urine. Urine sediment was obtained by centrifuging 5 mL of urine for 10 mins at 900 x g, removing 4.5 ml of supernatant, and resuspending the remaining 0.5 mL of urine. A 12 µl sample of the resuspended urine was microscopically assessed. The mean number of red blood cells and white blood cells per high power field (hpf) at 40 $\times$ magnification was expressed. Urine sediment with bacteriuria, and/or $>$5 red blood cells/hpf and/or $>$5 white blood cells/hpf, was considered active.

2.4. Bacteriological analyses

Urine samples were divided into 2 aliquots, one for the bacterial count and one for the bacterial culture. For the count, 1 ml of the room temperature urine sample was subjected to serial dilution ranging from 10^(−1) to 10^(−8) in 9 ml of sterile water. Subsequently, 1 ml of each dilution was plated on plate count agar media (PCA) (Liofilchem, Teramo, Italy) and incubated at 37 °C for 24 h to determine the Colony Forming Unit per milliliter (CFU /ml).

For bacterial culture, 2 ml of urine underwent centrifugation for 5 mins at 3000 $\times g$. The sediment was plated on Columbia Blood Agar (CBA), MacConkey Agar (MCK), Mannitol Salt Agar (MSA) and Tryptic Soy Broth (TSB) (Liofilchem, Teramo, Italy). All the cultural media were incubated for 48 h at 37 °C under aerobic conditions.

Blood samples were divided into three aliquots, with at least 1 ml designated for aerobic and anaerobic cultures and 100 µl for biomolecular examination.

For aerobic culture, 0.5 ml of blood samples were inoculated in 4.5 ml of Hemo-Aerobic culturing broth (Liofilchem, Teramo, Italy) and incubated at 37 °C for 48 h. The broth was then sub-cultured in CBA, MCK, and MSA (Liofilchem, Teramo, Italy) for 24 h. For anaerobic culture, 0.5 ml of blood samples were inoculated in 4.5 ml of the Hemo-Anaerobic Culturing broth (Liofilchem, Teramo, Italy) and incubated at 37 °C for at least 48 h. Subsequently, the broth was sub-cultured into

CBA for an additional 48 h. Bacterial identification was performed using Gram stain, biochemical micro-method tests (oxidase, catalase, and coagulase tests), and macro-method tests (API tests, Biomerieux, France).

2.5. Biomolecular analysis of blood samples

For the biomolecular analysis of blood samples, nucleic acid isolation was conducted using the IndiSpin Pathogen Kit (Indical Bioscience, Qiagen, Leipzig, Germany) according to the manufacturer's instructions. The isolated nucleic acid was then subjected to PCR targeting the 16S rRNA gene with universal primers BV5 and AV6 (Stecher et al., 2010). The primer sequences were as follows: BV5 5′-ATT AGA TAC CCY GGT AGT CC-3′ (tm 55 °C) and AV6 5′ ACG AGT GAC GAC ARC CAT G-3′ (tm 69.8 °C). An internal positive control and sterile water as a negative control were included in the PCR. The cycling conditions were as follows: 95 °C for 10 mins; 35 cycles of (94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s); and a final extension at 72 °C for 8 mins. Reaction conditions (50 µl) comprised 50 ng template DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM Mg2+, 0.2 mM dNTPs, 40 pmol of each primer, and 5 U of Taq DNA polymerase (AmpliTaq Gold™, Thermofisher, Italy). The PCR-amplified products (300 bp) underwent purification using the QIA-quick PCR Purification Kit (Qiagen, USA). Sequence analysis was performed using MiSeq NGS (Illumina, San Diego, California). The sequences were analyzed with Geneious v. 10.1 and compared with reference sequences available on the BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results

3.1. Study population

During the study period, 62 dogs and 60 cats were initially evaluated. Fifteen dogs were excluded (i.e., $n = 5$ incomplete data; n = 5 treated with antimicrobials within the previous 4 weeks; $n = 2$ concomitant hyperadrenocorticism; $n = 1$ previous urethrostomy surgery; n = 1 concurrent prostatic abscess; n = 1 concurrent urolithiasis). Nineteen cats were also excluded (i.e., $n = 6$ incomplete data; $n = 4$ treated with antimicrobials within the previous 4 weeks; $n = 3$ concomitant neurogenic bladder; n = 2 previous urethrostomy surgery; n = 1 concomitant feline immunodeficiency virus infection; n = 1 concurrent urolithiasis; n = 1 concomitant feline leukemia virus associated lymphoma; n = 1 treated with steroids within the previous 4 weeks). Forty-seven dogs and forty-one cats were finally enrolled in the study.

Information regarding the age, sex, and breed of dogs and cats, categorized by the CKD IRIS stages is shown in Table 1 and Table 2. The supplementary material contains all laboratory test data for the animals enrolled in the study (Tables S1-S8).

3.2. Dog population

Two out of 47 dogs (one in IRIS stage 3 and one in IRIS stage 4) presented bacteremia, as identified by positive blood cultures and biomolecular analysis, both with a final diagnosis of sepsis after the fulfilment of SIRS criteria. *Serratia marcescens* was identified in blood samples from both dogs. Additionally, one of these dogs had a positive urine culture (PUC) with the same pathogen isolated. The data on the bacteriological analysis, the haematological values and the SIRS criteria for dogs with bacteraemia are presented in Table 3. Forty-five out of the 47 dogs tested negative for bacteraemia, as confirmed by both blood cultures and PCR targeting 16S rRNA. Positive urine culture was found in further 7 dogs, 6 in a subclinical form and one with UTI presenting stranguria and pollakiuria. A total of 8 out of 47 (17%) dogs affected by CKD presented PUC with a single bacterial species isolated. (i.e., $n = 5$ *Escherichia coli*, $n = 1$ *Staphylococcus pseudintermedius*, n = 1 *Pseudomonas aeuriginosa*, and n = 1 *Serratia marcescens*).

Table 1
Demographic and urinalysis data for the total cohort according to the IRIS stage in the dog population.

	Total Cohort ($n = 47$)	IRIS stage 1 ($n = 7$)	2 ($n = 9$)	3 ($n = 14$)	4 ($n = 17$)
Demographic data[a]					
Age (years)	9 ± 3.5	8.8 ± 3.5	10.4 ± 4.0	8.5 ± 2.6	8.9 ± 4.0
Sex					
Male	22	5	4	9	4
Female	25	2	5	5	13
Breed					
Mixed	12	3	2	2	5
Other breeds	35	4	7	12	12
Urinalysis data[a]					
Positive Urine Culture	8	1	1	4	2
USG					
≤1.012	1	0	0	0	1
1.013–1.034	7	1	1	4	1
≥1.035	0	0	0	0	0
AUS					
Yes	7	0	1	4	2
No	1	1	0	0	0
Negative Urine Culture	39	6	8	10	15
USG					
≤1.012	7	0	2	1	4
1.013–1.034	28	3	5	9	11
≥1.035	4	3	1	0	0
AUS					
Yes	8	2	2	2	2
No	31	4	6	8	13

Abbreviation: IRIS, International renal interest society; USG, urine specific gravity; AUS, active urine sediment.
[a] Table entries are reported as: Mean and Standard Deviation (M ± SD) for continuous variables, and proportions for categorical variables.

Urine culture results according to IRIS stage, urinalysis data, and clinical presentation in dogs with bacteriuria are reported in Table 1 and Table 4.

3.3. Cat population

One out of 41 cats (IRIS stage 1) had bacteremia, as identified by positive blood cultures and biomolecular analysis, not associated with SIRS and with negative urine culture. The bacterial species isolated in the blood sample was *Enterococcus* spp. The data on the bacteriological analysis, the haematological values and the SIRS criteria for the cat with bacteremia are presented in Table 3. Forty of the 41 cats did not have bacteremia, as confirmed by negative blood cultures and PCR targeting 16S rRNA. Positive urine cultures were detected in 6 out of 41 (15%) cats with one of them presenting periuria being classified as affected by UTI. A single bacterial species was isolated in 5 out of 6 cats with PUC [i.e., *Escherichia coli* ($n = 2$), *Enterococcus faecalis* (n = 2) and *Streptococcus* spp. (n = 1)], whereas 2 isolates were found in one cat (i.e., *Staphylococcus hominis* and *Micrococcus* spp).

Urine culture results according to IRIS stage, urinalysis data, and clinical presentation in cats with bacteriuria are reported in Table 2 and Table 4.

4. Discussion

This study shows a low prevalence of bacteremia in dogs and cats affected by CKD. Indeed, only two out of 47 dogs and one out of 41 cats presented bacteremia. To date, with the exception on *Bartonella* spp. infections in cats, few data are available regarding bacteremia in

Table 2
Demographic and urinalysis data for the total cohort and according to the IRIS stage in the cat population.

	Total Cohort (n = 41)	IRIS stage			
		1 (n = 14)	2 (n = 11)	3 (n = 9)	4 (n = 7)
Demographic data[a]					
Age (years)	6.84 ± 4.43	5.32 ± 4.52	9.86 ± 5.07	6.11 ± 2.15	6.06 ± 3.72
Sex					
Male	24	6	6	7	5
Female	17	8	5	2	2
Breed					
Mixed	29	11	9	4	5
Other breeds	12	3	2	5	2
Urinalysis data[a]					
Positive Urine Culture	6	3	1	1	1
USG					
≤1.012	0	0	0	0	0
1.013–1.034	5	2	1	1	1
≥1.035	1	1	0	0	0
AUS					
Yes	4	3	1	0	0
No	2	0	0	1	1
Negative Urine Culture	35	11	10	8	6
USG					
≤1.012	5	0	1	1	3
1.013–1.034	25	8	7	7	3
≥1.035	5	3	2	0	0
AUS					
Yes	8	2	1	4	1
No	27	9	9	4	5

Abbreviation: IRIS, International renal interest society; USG, urine specific gravity; AUS, active urine sediment.
[a] Table entries are reported as: Mean and Standard Deviation (M ± SD) for continuous variables, and proportions for categorical variables.

companion animals, with most available information coming from sick patients with suspected sepsis (Greiner et al., 2008). To the best of the authors' knowledge, this is the first insight into the prevalence of bacteremia in a cohort of dogs and cats affected by CKD, a recognized risk factor for bloodstream infections in human medicine (Rojas et al., 2013; Dagasso et al., 2020).

Bacteria may gain access to the bloodstream from densely colonized mucosal surfaces or localized infection sites within the body. The conditions most frequently associated with bacteremia are malignant neoplasms and infections of the skeletal, cardiovascular, and urogenital systems (Hirsh et al., 1984). However, sepsis only develops in patients with compromised immune defence, or when the host defences are overwhelmed by the number of bacteria entering the bloodstream (Goodwin and Schaer, 1989). In the CKD population herein considered, out of 3 animals with bacteremia (i.e., two dogs and one cat), only dogs presented sepsis. Moreover, both dogs were affected by advanced CKD with uremia while the cat had stage 1 CKD. Therefore, it is reasonable to suppose that uremia could have contributed to the onset of sepsis. Indeed, the accumulation of uremic toxins can inhibit the activation and disrupt the phagocytic functions of polymorphonuclear leukocytes (PMNLs). This condition, together with enhanced apoptosis, causes a decreased immune response which characterized the uremia (Cohen and Hörl, 2012).

Additionally, no instances of bacteriuria-related bacteriemia were observed in the cat population herein considered and only one out of the 47 dogs enrolled received a diagnosis of urosepsis, which was confirmed by the identification of SIRS along with the isolation of the same pathogen (*S. marcescens*) in urine and blood cultures. In human medicine, bacteriuria represents the primary source of bloodstream infections and subsequent sepsis in patients affected by mild to severe CKD. The UTI

Table 3
Bacteriological, haematological and SIRS criteria data for dogs and cats with bacteraemia.

Dog population	IRIS Stage	Bacteremia		Bacteriuria	CBC					APPs		SIRS criteria		
		Blood culture	PCR 16 s RNA		RBC 5.5–8.0 (x 1000/μL)	WBC 6,0–14 (x 1000/μL)	Bands 0–300 (/μL)	Segs 3500–9300 (/μL)	Lymphs 1200–3200 (/μL)	Alb 2.8–3.7 (g/dL)	CRP 0.01–0.45 (mg/dL)	BT	HR	PP
ID														
DOG35	III	*Serratia marcescens*	Positive	*Serratia marcescens*	1.91	32.8	0	29000	1900	1.4	12.73	High	Normal	Present
DOG43	IV	*Serratia marcescens*	Positive	Culture-negative	5.71	7.2	0	6100	700	3.2	13.21	Low	Low	Absent
Cat population	IRIS Stage	Bacteremia		Bacteriuria	CBC					APPs		SIRS criteria		
		Blood culture	PCR 16 s RNA		RBC 6.0–9.5 (x 1000/μL)	WBC 5,5–12 (x 1000/μL)	Bands 0–300 (/μL)	Segs 3000–7000 (/μL)	Lymphs 1300–5500 (/μL)	Alb 2.8–3.7 (g/dL)	SAA 0.1–0.5 (ug/mL)	BT	HR	PP
ID														
CAT12	I	*Enterococcus* spp.	Positive	Culture-negative	6.9	11.8	0	9400	1200	3.3	0.7	Normal	Normal	Absent

Abbreviation: IRIS, International Renal Interest Society; CBC, complete blood count; APPs, acute phase proteins; SIRS, systemic inflammatory response syndrome; RBC, red blood cells; WBC, white blood cells; Bands, band neutrophils; Segs, segmented neutrophils; Lymphs, lymphocytes; Alb, serum albumin; CRP, C-reactive protein; SAA, serum amyloid A; BT, body temperature; HR, heart rate; PP, polypnea.

Table 4
Data on urine culture, urinalysis, and clinical presentation in dogs and cats presenting bacteriuria.

ID	IRIS Stage	Urine culture		Urinalysis data						Clinical presentation
		Bacterial species	Microbial count	USG	pH	RBC/hpf	WBC/hpf	Bacteria	Urine sediment	
Dog population										
DOG3	III	*Escherichia coli*	$> 10^3$ CFU/ml	1022	6	10	5	Absent	Active	SB
DOG4	III	*Escherichia coli*	$> 10^3$ CFU/ml	1017	6,5	10	50	Bacilli+++	Active	SB
DOG7	I	*Staphylococcus pseudintermedius*	nd*	1014	6	<5	<5	Absent	Inactive	SB
DOG9	III	*Pseudomonas aeruginosa*	nd*	1014	6	<5	20	Bacilli+	Active	SB
DOG11	IV	*Escherichia coli*	nd*	1012	6	5	10	Absent	Active	SB
DOG21	IV	*Escherichia coli*	10^6 CFU/ml	1020	7	10	50	Bacilli++	Active	SB
DOG35	III	*Serratia marcescens*	10^3 CFU/ml	1016	6,5	30	50	Bacilli++	Active	UTI
DOG37	II	*Escherichia coli*	10^6 CFU/ml	1019	6	20	20	Bacilli++	Active	SB
Cat population										
CAT1	I	*Escherichia coli*	$> 10^5$ CFU/ml	1020	6,5	<5	10	Bacilli++	Active	SB
CAT2	II	*Streptococcus* spp.	$> 10^3$ CFU/ml	1015	6	<5	<5	Absent	Inactive	SB
CAT13	I	*Escherichia coli*	$> 10^1$ CFU/ml	1035	7	10	<5	Absent	Active	SB
CAT14	I	*Staphylococcus hominis* + *Micrococcus* spp.	nd*	1022	6,5	50	<5	Absent	Active	UTI
CAT33	IV	*Enterococcus faecalis*	$> 10^9$ CFU/ml	1018	6	5	5	Absent	Inactive	SB
CAT36	III	*Enterococcus faecalis*	3×10^7 CFU/ml	1024	7	5	10	Cocci ++	Active	SB

Abbreviation: IRIS, International Renal Interest Society; USG, urine specific gravity; RBC/hpf, red blood cells per high power field; WBC/hpf, white blood cells per high power field; CFU/ml, colony forming unit per milliliter; nd, not determined; SB, subclinical bacteriuria; UTI, urinary tract infection.
nd*: microbial count not determined due to insufficient sample size.

evolution into systemic infections is attributed to immunological, metabolic, and inflammatory disorders hindering bacteria clearance and triggering uropathogen proliferation (Scherberich et al., 2021). Though scant data are available from the scientific literature, a similar relationship between CKD, bacteriuria and bloodstream infections could be hypothesized in veterinary medicine. Indeed, previous evidence suggest that CKD leads to a systemic inflammatory status in cats (Uva et al., 2023) and an immune system impairment in both dogs and cats (Keegan and Webb, 2010; Bosco et al., 2017). These conditions, along with the high prevalence of bacteriuria observed in both dogs and cats affected by CKD (Mayer-Roenne et al., 2007; White et al., 2013; Foster et al., 2018; Lamoureux et al., 2019; Hindar et al., 2020; Uva et al., 2022) could predispose these patients to bacteremia. Moreover, CKD has been listed among the most common preexisting conditions in dogs diagnosed with urosepsis resulting from varying causes (i.e., pyometra, prostatitis, or pyelonephritis) (Perry et al., 2022). Nevertheless, it remained uncertain whether the link between CKD and urosepsis was causal or merely associative (Perry et al., 2022). However, the low prevalence of bacteremia and bacteriuria-related bacteremia described in the present study fails to confirm this hypothesis. This finding agrees with a previous study involving a population of cats with neurogenic bladder in which, unlike in humans, bacteriuria did not result in systemic infection (Uva et al., 2022). None of the animals enrolled in the present study had experienced prolonged hospitalization or urinary catheterization, which are recognized as potential risk factors for urosepsis in humans, nor were affected by comorbidities commonly associated with systemic infections in humans with CKD, such as diabetes mellitus or pelvic malignancy (Dimitrijevic et al., 2021).

The bacterial species identified in the two dogs presenting bacteremia and sepsis, both affected by advanced stage CKD with uremia, was *S. marcescens*, a ubiquitous motile Gram-negative bacillus. Additionally, one of these dogs presented bacteriuria with the same pathogen isolated. This bacterium is rarely associated with UTIs in dogs (Loncaric et al., 2020), except for one case report where it was found in the blood and urine cultures of a dog with systemic clinical signs unresponsive to previous antibiotic therapy (Broy et al., 1997). Considering the opportunistic nature of *S. marcescens*, it is possible to assume that the uremic condition of the dogs contributed to the evolution of the infection into sepsis.

Concerning bacteriuria, the available data indicate an increased occurrence of PUC in dogs and cats with CKD (Mayer-Roenne et al., 2007; White et al., 2013; Foster et al., 2018; Lamoureux et al., 2019; Hindar et al., 2020; Uva et al., 2022). The clinical presentation of bacteriuria in both dogs and cats, including those affected by CKD, varies from subclinical bacteriuria (SB) (i.e., the presence of PUC in the absence of clinical manifestations of lower urinary tract disease) (Weese et al., 2019) to UTI (i.e., the presence of PUC accompanied by an inflammatory response and clinical manifestations such as gross hematuria, stranguria, pollakiuria and periuria) (Wood, 2017). Consistent with previous findings, a high prevalence rate of PUC in dogs (i.e., 17%) and cats (i.e., 15%) affected by CKD has been herein detected, with SB being the most frequent clinical presentation observed in our population.

Currently, the clinical relevance of SB in patients affected by CKD remains unclear, and there is a need for more definitive guidance on its ideal treatment. Actually, treatment of SB with antimicrobials is rarely indicated and discouraged. Indeed, the current International Society for Companion Animal Infectious Diseases (ISCAID) guidelines recommend treatment of SB only for a few urinary conditions, which do not include CKD (Weese et al., 2019). However, investigating the long-term outcomes for dogs and cats with CKD and concurrent SB may provide valuable information about the occurrence of UTIs and their potential evolution toward systemic infections and urosepsis. This is a critical issue as bacterial urinary tract disease ranks among the primary causes of over prescription of antimicrobials in dogs and cats (Hardefeldt et al., 2018). An improper approach to infection management in companion animals can pose a significant threat to global Public Health. Indeed, evidence that antimicrobial use in animals may contribute to some multidrug-resistant bacterial infections in humans is increasing

(Guardabassi et al., 2018).

Although in the present study, one-time blood sampling to assess bacteremia in enrolled patients was used instead of three blood samples taken one hour apart (Willems et al., 2012), biomolecular analysis was performed on all blood samples to increase the likelihood of detecting bacteremia (Florio et al., 2018). In this regard, it is worth noting that the outcomes of biomolecular analysis were consistent with the results derived from blood culture analysis.

5. Conclusions

In conclusion, this study provides preliminary results on the occurrence of bacteremia in dogs and cats affected by CKD, demonstrating a low prevalence in both species examined. Furthermore, this study confirms a high prevalence of bacteriuria in companion animals with CKD. Finally, within our study population, only one dog and no cats presented bacteriuria-related bacteremia. This is the first-time data have been obtained from concomitant blood and urine cultures executed in the entire population of enrolled dogs and cats.

Ethics statement

This study was approved by the Ethics Committee of the Department of Veterinary Medicine of Bari, Italy (Approval number, Prot. Uniba 25/2021).

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CRediT authorship contribution statement

Annamaria Uva: Writing – original draft, Investigation, Data curation, Conceptualization. **Maria Alfonsa Cavalera:** Writing – review & editing, Data curation. **Floriana Gernone:** Writing – review & editing. **Souad Nasar:** Writing – review & editing. **Paola Ghergo:** Writing – review & editing, Investigation. **Marco Cordisco:** Writing – review & editing, Investigation, Data curation. **Marialaura Corrente:** Writing – review & editing, Supervision, Conceptualization. **Andrea Zatelli:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2024.105382.

References

Bosco, A.M., Almeida, B.F.M., Pereira, P.P., Dos Santos, D.B., Neto, Á.J.S., Ferreira, W.L., Ciarlini, P.C., 2017. The uremic toxin methylguanidine increases the oxidative metabolism and accelerates the apoptosis of canine neutrophils. Vet. Immunol. Immunopathol. 185, 14–19. https://doi.org/10.1016/j.vetimm.2017.01.006.

Broy, P., Diquélou, A., Raymond-Letron, I., Dossin, O., 1997. Serratia Marcescens bacteremia in a dog. Rev. Med. Veterinaire 148, 445–450.

Cohen, G., Hörl, W., 2012. Immune dysfunction in uremia—an update. Toxins 4, 962–990. https://doi.org/10.3390/toxins4110962.

Dagasso, G., Conley, J., Steele, L., Parfitt, E.E.C., Pasquill, K., Laupland, K.B., 2020. Risk of bloodstream infection in patients with renal dysfunction: a population-based cohort study. Epidemiol. Infect. 148, e105 https://doi.org/10.1017/S0950268820001041.

Dimitrijevic, Z., Paunovic, G., Tasic, D., Mitic, B., Basic, D., 2021. Risk factors for urosepsis in chronic kidney disease patients with urinary tract infections. Sci. Rep. 11, 14414. https://doi.org/10.1038/s41598-021-93912-3.

Dorsch, R., Teichmann-Knorrn, S., Lund, H., 2019. Urinary tract infection and subclinical bacteriuria in cats a clinical update. J. Feline Med. Surg. 21, 1023–1038. https://doi.org/10.1177/1098612X19880435.

Florio, W., Morici, P., Ghelardi, E., Barnini, S., Lupetti, A., 2018. Recent advances in the microbiological diagnosis of bloodstream infections. Crit. Rev. Microbiol. 44, 351–370. https://doi.org/10.1080/1040841X.2017.1407745.

Foster, J.D., Krishnan, H., Cole, S., 2018. Characterization of subclinical bacteriuria, bacterial cystitis, and pyelonephritis in dogs with chronic kidney disease. J. Am. Vet. Med. Assoc. 252, 1257–1262. https://doi.org/10.2460/javma.252.10.1257.

Goodwin, J.K., Schaer, M., 1989. Septic shock. Vet. Clin. North Am. Small Anim. Pract. 19, 1239–1258. https://doi.org/10.1016/s0195-5616(89)50137-2.

Greiner, M., Wolf, G., Hartmann, K., 2008. A retrospective study of the clinical presentation of 140 dogs and 39 cats with bacteraemia. J. Small Anim. Pract. 49, 378–383. https://doi.org/10.1111/j.1748-5827.2008.00546.x.

Guardabassi, L., Apley, M., Olsen, J.E., Toutain, P.-L., Weese, S., 2018. Optimization of antimicrobial treatment to minimize resistance selection. Microbiol. Spectr. 6 https://doi.org/10.1128/microbiolspec.ARBA-0018-2017.

Hardefeldt, L.Y., Selinger, J., Stevenson, M.A., Gilkerson, J.R., Crabb, H., Billman-Jacobe, H., Thursky, K., Bailey, K.E., Awad, M., Browning, G.F., 2018. Population wide assessment of antimicrobial use in dogs and cats using a novel data source - a cohort study using pet insurance data. Vet. Microbiol. 225, 34–39. https://doi.org/10.1016/j.vetmic.2018.09.010.

Hindar, C., Chang, Y., Syme, H.M., Jepson, R.E., 2020. The association of bacteriuria with survival and disease progression in cats with azotemic chronic kidney disease. J. Vet. Intern. Med. 34, 2516–2524. https://doi.org/10.1111/jvim.15918.

Hirsh, D.C., Jang, S.S., Biberstein, E.L., 1984. Blood culture of the canine patient. J. Am. Vet. Med. Assoc. 184, 175–178.

IRIS staging guidelines of CKD, 2023. http://www.iris-kidney.com accessed 12.1.23.

Ishigami, J., Matsushita, K., 2019. Clinical epidemiology of infectious disease among patients with chronic kidney disease. Clin. Exp. Nephrol. 23, 437–447. https://doi.org/10.1007/s10157-018-1641-8.

Keegan, R.F., Webb, C.B., 2010. Oxidative stress and neutrophil function in cats with chronic renal failure: oxidative stress in renal failure. J. Vet. Intern. Med. 24, 514–519. https://doi.org/10.1111/j.1939-1676.2010.0498.x.

Lamoureux, A., Da Riz, F., Cappelle, J., Boulouis, H.-J., Benchekroun, G., Cadoré, J.-L., Krafft, E., Maurey, C., 2019. Frequency of bacteriuria in dogs with chronic kidney disease: a retrospective study of 201 cases. J. Vet. Intern. Med. 33, 640–647. https://doi.org/10.1111/jvim.15434.

Loncaric, I., Misic, D., Szostak, M.P., Künzel, F., Schäfer-Somi, S., Spergser, J., 2020. Broad-Spectrum cephalosporin-resistant and/or fluoroquinolone-resistant Enterobacterales associated with canine and feline urogenital infections. Antibiotics 9, 387. https://doi.org/10.3390/antibiotics9070387.

Marino, C.L., Lascelles, B.D.X., Vaden, S.L., Gruen, M.E., Marks, S.L., 2014. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. J. Feline Med. Surg. 16, 465–472. https://doi.org/10.1177/1098612X13511446.

Mayer-Roenne, B., Goldstein, R.E., Erb, H.N., 2007. Urinary tract infections in cats with hyperthyroidism, diabetes mellitus and chronic kidney disease. J. Feline Med. Surg. 9, 124–132. https://doi.org/10.1016/j.jfms.2006.09.004.

O'Neill, D.G., Elliott, J., Church, D.B., McGreevy, P.D., Thomson, P.C., Brodbelt, D.C., 2013. Chronic kidney disease in dogs in UK veterinary practices: prevalence, risk factors, and survival. J. Vet. Intern. Med. 27, 814–821. https://doi.org/10.1111/jvim.12090.

Perry, K.M., Lynch, A.M., Caudill, A., Vigani, A., Roberston, J.B., Vaden, S., 2022. Clinical features, outcome, and illness severity scoring in 32 dogs with urosepsis (2017–2018). J. Vet. Emerg. Crit. Care 32, 236–242. https://doi.org/10.1111/vec.13158.

Polzin, D.J., 2011. Chronic kidney disease in small animals. Vet. Clin. North Am. Small Anim. Pract. 41, 15–30. https://doi.org/10.1016/j.cvsm.2010.09.004.

Rojas, L., Muñoz, P., Kestler, M., Arroyo, D., Guembe, M., Rodríguez-Créixems, M., Verde, E., Bouza, E., 2013. Bloodstream infections in patients with kidney disease: risk factors for poor outcome and mortality. J. Hosp. Infect. 85, 196–205. https://doi.org/10.1016/j.jhin.2013.07.009.

Scherberich, J.E., Fünfstück, R., Naber, K.G., 2021. Urinary tract infections in patients with renal insufficiency and dialysis – epidemiology, pathogenesis, clinical symptoms, diagnosis and treatment. GMS. Infect. Dis. 9, Doc07. https://doi.org/10.3205/id000076.

Spillane, A.M., Haraschak, J.L., Gephard, S.E., Nerderman, B.E., Fick, M.E., Reinhart, J. M., 2023. Evaluating the clinical utility of the systemic inflammatory response syndrome criteria in dogs and cats presenting to an emergency department. J. Vet. Emerg. Crit. Care 33, 315–326. https://doi.org/10.1111/vec.13293.

Stecher, B., Chaffron, S., Käppeli, R., Hapfelmeier, S., Freedrich, S., Weber, T.C., Kirundi, J., Suar, M., McCoy, K.D., Mering, C., Macpherson, A.J., Hardt, W.-D., 2010. Like will to like: abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria. PLoS Pathog. 6, e1000711 https://doi.org/10.1371/journal.ppat.1000711.

Uva, A., Gernone, F., Cavalera, M.A., Carelli, G., Cordisco, M., Trotta, A., Donghia, R., Corrente, M., Zatelli, A., 2022. Prevalence of bacteriuria in cats with neurogenic bladder. Vet. Res. Commun. 46, 1075–1084. https://doi.org/10.1007/s11259-022-09973-w.

Uva, A., Cavalera, M.A., Gusatoaia, O., Donghia, R., Gernone, F., Silvestrino, M., Zatelli, A., 2023. Inflammatory status and chronic kidney disease in cats: old and

new inflammatory markers-a pilot prospective study. Anim. Open Access J. MDPI 13, 3674. https://doi.org/10.3390/ani13233674.

Weese, J.S., Blondeau, J., Boothe, D., Guardabassi, L.G., Gumley, N., Papich, M., Jessen, L.R., Lappin, M., Rankin, S., Westropp, J.L., Sykes, J., 2019. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. Vet. J. Lond. Engl. 1997 (247), 8–25. https://doi.org/10.1016/j.tvjl.2019.02.008.

White, J.D., Stevenson, M., Malik, R., Snow, D., Norris, J.M., 2013. Urinary tract infections in cats with chronic kidney disease. J. Feline Med. Surg. 15, 459–465. https://doi.org/10.1177/1098612X12469522.

Willems, E., Smismans, A., Cartuyvels, R., Coppens, G., Van Vaerenbergh, K., Van den Abeele, A.-M., Frans, J., Bilulu Study Group, 2012. The preanalytical optimization of blood cultures: a review and the clinical importance of benchmarking in 5 Belgian hospitals. Diagn. Microbiol. Infect. Dis. 73, 1–8. https://doi.org/10.1016/j.diagmicrobio.2012.01.009.

Wood, M.W., 2017. Lower urinary tract infections. In: Ettinger, S.J., Feldman, E.C., Côté, E. (Eds.), Textbook of Veterinary Internal Medicine. Elsevier, St. Louis, MO, St. Louis, MO, pp. 1992–1996.

Xu, H., Gasparini, A., Ishigami, J., Mzayen, K., Su, G., Barany, P., Ärnlöv, J., Lindholm, B., Elinder, C.G., Matsushita, K., Carrero, J.J., 2017. eGFR and the risk of community-acquired infections. Clin. J. Am. Soc. Nephrol. 12, 1399–1408. https://doi.org/10.2215/CJN.00250117.