Clinical and microbiological characterization of subclinical bacteriuria and sporadic bacterial cystitis in dogs with spontaneous hypercortisolism

Letícia Machado a, Milena Cleff de Oliveira b, Cláudia Ruga Barbieri b, Camila Império Riboldi b, Vanessa Bielefeldt Leotti c, Félix Hilário Díaz González c, d, Stella de Faria Valle a, d, Franciele Maboni Siqueira a, d, Alão Gomes Pöppl a, d, *

a Veterinary Sciences Post-Graduation Program, Federal University of Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, Rio Grande do Sul, CEP 91540-000, Brazil
b Faculty of Veterinary Medicine, Federal University of Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, Rio Grande do Sul, CEP 91540-000, Brazil
c Department of Statistics, Mathematics and Statistics Institute, Federal University of Rio Grande do Sul, Av. Bento Gonçalves 9500, Porto Alegre, Rio Grande do Sul, CEP 91540-000, Brazil
d Department of Veterinary Clinical Pathology, School of Veterinary Medicine, Federal University of Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, Rio Grande do Sul, CEP 91540-000, Brazil

ABSTRACT

Study’s aims were to characterize subclinical bacteriuria (SB) and sporadic bacterial cystitis (SBC) in dogs with spontaneous hypercortisolism (HC). Prospective cross-sectional design divided patients as newly diagnosed (n = 27), poorly controlled (n = 21), well controlled (n = 34), and controls (n = 19). Urine culture positive results were identified by MALDI-TOF and submitted to antibiogram. Escherichia coli was the most common microorganism (36%). The majority of positive cultures in HC were SB (12.2%). All 4.1% SBC cases were in well controlled HC cases. Bacteriuria correlated with low urine specific gravity and low lymphocyte count. HC degree of control correlated with leukocyturia. SB/SBC cases were treated based in antimicrobial susceptibility leading to microbiological cure in 75% of HC cases. Persistent infections occurred only in SB cases, all by E. coli which became more resistant. SB/SBC prevalence in canine HC is actually lower. Further evidence for current ISCAID guideline contraindication for SB treatment due to HC were provided.

1. Introduction

Canine spontaneous hypercortisolism (HC) is characterized as a group of clinical signs resulting from excessive exposure to glucocorticoids (GC) effects [1,2]. It is a common disease in older dogs and considered one of the main canine endocrinopathy [3]. Patients with HC have an increased risk of developing clinical complications due to gluconeogenic, catabolic, anti-inflammatory, and immunosuppressive effects of GC [1].

Among these complications, the damage to the host’s defense mechanisms allows the adhesion of microorganisms in the urinary tract, as well as their multiplication and persistence, leading to urinary tract infection.
infections (UTI) [4]. Previous studies reported that approximately 39–50% of dogs with spontaneous HC or those under continuous use of GC presented concomitant UTI [5–7]. This UTI predisposition might be related to the inhibition of the neutrophils and macrophages migration to the affected areas induced by glucocorticoids [8]. Typical UTI clinical signs include pollakiuria, dysuria, and hematuria; however, many dogs with HC may remain asymptomatic possibly due to cortisol anti-inflammatory effects [1], as well as due to polyuria secondary to HC-induced ADH inhibition [1] that can make it difficult for tutors to detect clinical signs, and by these ways classified as presenting sub-clinical bacteriuria (SB) [9]. SB was defined by current ISCAID guidelines as the presence of bacteria in urine as determined by positive bacterial culture from a properly collected urine specimen, in the absence of clinical evidence of infectious urinary tract disease. In contrast, Sporadic bacterial cystitis (SBC) is a sporadic bacterial infection of the urinary bladder with compatible lower urinary tract signs [9].

Chronic lower UTI may increase the probability of ascending infections (pyelonephritis) and renal failure [10]. The identification of the etiologic agent is crucial for the establishment of an appropriate therapy [9,11] due to the growth of antimicrobials resistance, partly due to the extensive use of antimicrobial drugs in veterinary medicine [12] but also due to the closer contact between companion animals and people, which facilitates bacterial transmission [13]. Currently, because the greater knowledge and concern about HC, diagnosis tends occur earlier [14]; nevertheless, current data on the prevalence and bacterial profile of these UTI patients are scarce [15]. Moreover, concepts and classification of bacterial urinary infections have changed in the past years. While previous ISCAID Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats categorize bacterial cystitis in patients with comorbidities such HC as ‘complicated’ and supported antimicrobial treatment independently of the presence of clinical signs [11]; current ISCAID guidelines do not recommend antimicrobial treatment in patients with HC and SB [9].

The aims of this study were to evaluate the prevalence of SB and SBC in dogs with spontaneous HC recently diagnosed HC or in current medical treatment, and to characterize clinical, laboratory, and microbiological aspects of UTI, including the pattern of antimicrobials susceptibility of bacteria isolated from dogs with HC, therapeutic follow-up after antimicrobial treatment, and eventual differences between bacteria profile from dogs with HC and dogs with UTI without HC.

2. Material and methods

2.1. Animals and group allocation

A prospective cross-sectional study was conducted between January 2018 and December 2018 with dogs diagnosed with HC. Patients were selected based on the presence of compatible history, clinical, and laboratory signs; and with HC diagnosis confirmed by a positive low dose dexamethasone suppression test (LDDST) or ACTH stimulation test. There were included dogs currently in treatment with trilostane and newly diagnosed. One hundred and one samples from 74 different dogs (82 samples from 55 dogs with HC and 19 without HC) were included in the study.

Patients with HC were distributed into three groups: Group 1 – HC newly diagnosed without treatment (NDHC, $n = 27$); Group 2 – HC under medical treatment but poor clinical control (PCHC, $n = 21$) and Group 3 – HC under medical treatment with good clinical control (WCHC, $n = 34$).

A fourth group of dogs without HC but presenting clinical signs, urinalysis, or ultrasound images compatible with bladder inflammation (e.g., thickening and/or irregularities in bladder wall, active sediment) was included as control ($n = 19$) to verify eventual difference between bacterial profile of dogs with HC or without. Hormonal tests were not performed in the Control group patients, since they did not present history, physical abnormalities, or laboratorial evidences of HC [14].

Concerning the dogs with HC, 22 participated more than once in the study, when it was found that the degree of HC control has changed when compared to first participation, provided that a minimum interval of three months was respected between the different evaluations. Among these, 11 were from the NDHC group, who participated in the survey again after starting treatment with trilostane and returning for reevaluation. The other 11 dogs were relocated between the WCHC and PCHC groups after reevaluation. Patients with a positive result in urine culture were not reclassified, despite the degree of HC control.

Dogs with HC were categorized according to the first laboratorial analysis. Treated dogs who showed little or no improvement in hypercortisolism’s clinical signs or laboratory tests, and post-ACTH cortisol $>7.0$ μg/dL, were classified as poorly controlled and were allocated to the PCHC group. Dogs in treatment, who had showed clear improvement in clinical signs and laboratory tests and have a post-ACTH cortisol $<7.0$ μg/dL, were classified as presenting HC good control, and allocated to the WCHC group. Patients with history, clinical, and laboratorial features compatible with HC were included in the NDHC group after confirming diagnosis by LDDST or ACTH stimulation test [14].

The use of systemic antimicrobials in the last month or coexistence of other UTI predisposing conditions (i.e., diabetes mellitus, urolithiasis, urinary bladder tumor, spinal cord lesion) were adopted as exclusion criteria. The study was approved by the Ethics Committee on the Use of Animals at the Federal University of Rio Grande do Sul (32522).

2.2. Patient’s data base

Blood samples were collected in to EDTA-K$_2$ and without anticoagulant tubes after 10–12 h fasting. Complete blood count (ProCyte Dx, Idexx Laboratories) and serum biochemistry (ALP, ALT, creatinine, urea, glucose, total cholesterol, and triglycerides) were performed using an automatic analyzer (CM 200, Wiener Lab, Argentina). These tests were used to classify the degree of control in patients with HC, along with the clinical and hormonal evaluation, as well as to document absence of laboratory evidence of HC in the control group.

Urine samples were obtained via ultrasound-guided cystocentesis after hair clipping from the ventral abdomen and routine asepsis with 2% chlorhexidine and 70% isopropyl alcohol. Ultrasound gel was substituted by isopropyl alcohol. The samples were collected in 10 mL syringes and a volume of 0.5 mL was immediately aliquoted into sterile tubes to culture and antibiogram. Urinalysis was performed considering physical (turbidity, color, and urine specific gravity), chemical (pH, protein, glucose, ketones, bilirubin), and sediment examination after centrifugation (optical microscopy in 400x magnification). Urine specific gravity (USG) was determined by refractometry after centrifugation. Dogs with discrete proteinuria by dipstick test (> 30 mg/dL), had the urinary protein to creatinine ratio (UPC) evaluated using an automatic analyzer (CM 200, Wiener Lab, Argentina).

All ultrasonographic examinations were performed using either a linear 7.5–12 MHz or a curved 5.5–8 MHz electric array transducer and a My Lab™80 ultrasound system (Esaote, Italy). Dogs were scanned to examine the entire abdomen with particular emphasis being placed on the urinary tract and on adrenal glands in order to analyze possible changes that could suggest UTI, as well as to monitor changes in the adrenals and in other organs of patients with HC, respectively.

2.3. Hormonal tests

Clinical and laboratory parameters associated with post-ACTH cortisol were used to classify the degree of HC control of dogs undergoing drug therapy. The ACTH stimulation test consisted in the collection of blood for cortisol measurement after one hour of intravenous administration of synthetic ACTH, at a dose of 1 μg/kg [16]. All ACTH stimulation tests were performed between two and four hours after trilostane administration [17]. One of the dogs was being treated with mitotane. Serum samples were sent to a reference laboratory for cortisol
measurements and measured by radioimmunoassay (ImmuChem – MP Biomedicals, USA).

2.4. Microbiological tests and antimicrobial treatment

Urinary aliquots were immediately submitted for microbiological analysis after being collected from the dogs. Firstly, the samples were inoculated on 5% sheep blood agar plate (K25-610005, Kasvi, Brazil) and MacConkey agar (K25-610028, Kasvi, Brazil) and incubated at 37°C for up to 48 h in aerobic conditions. When bacterial growth was observed, the antimicrobial susceptibility profile was determined by Kirby-Bauer disc diffusion according to recommendations of Performance Standards for Antimicrobial Susceptibility Testing (CLSI, 2018) with the following drugs (NewProv, Brazil): cephalixin, amoxicillin with clavulanic acid, enrofloxacin, sulfamethoxazole with trimethoprim, doxycycline and nitrofurantoin. The breakpoints used for the interpretation were according to CLSI-VET08 (2018) [18] and BrCAST (2019) [19] guidelines. Escherichia coli ATCC 25922 and ATCC 35218 were used as quality control strains.

Bacterial identification was performed by MALDI-TOF (Matrix Associated Laser Desorption-Ionization - Time of Flight) [20] in a MALDI Biotype 4.0 Realtime Identification (Bruker Daltonics). The captured spectra were compared to the spectra library using the BioTyper 4.0 program (Bruker Daltonics). Scores above 2.0 were used for species assignment, and scores between 1.7 and 2.0 for gender level confirmation [20].

After bacterial growth documentation patients were treated based in their respective antimicrobial susceptibility profile for 15 days accordingly to previous ISCAID guidelines [11] at the time the study was done. All treated patients were followed up and submitted to urine sampling after seven days from antimicrobial treatment has ended as previously described for urinalysis and bacterial culture.

2.5. Statistical analysis

Quantitative variables were described using mean and standard deviation, as well as box diCRama. Qualitative variables were described through frequencies and percentages. Fisher’s exact test was used to compare the occurrence of the interest features (urinary infection, sensitivity to antimicrobials, and others) among groups. The level of significance adopted was 5%. The statistical package used was SPSS version 18.

3. Results

3.1. Animals

Most patients within the study were neutered females, aging between 4 and 16 years old. Demographic data of participants are showed in Table 1.

3.2. Microbiological results

Positive urine cultures were observed in 14.6% (12/82) of urine samples from dogs with HC and in 47.4% (9/19) of samples from the control group (Table 2). Proportion of positive cultures was significantly greater in the Control group (P = 0.024) in comparison with the HC groups, being PCHC group the one with smallest proportion of positive cultures. No statistical differences were detected between the number of positive cultures in the NDHC and WCHC groups.

Urine infections were predominantly by Gram-negative bacteria (60% of the isolates) in analyzed samples. Among 21 cases with positive urine culture, only one dog (4.7%) had mixed infection caused by Bacillus firmus and Staphylococcus capitis in the WCHC group, while single bacteria caused the remaining infections (Table 3). E. coli was the most prevalent microorganism in the positive urinary cultures, being present in 38% (8/21) of the samples. One bacterium could not be identified due to the lack of peaks detection in MALDI-TOF. There was no significant difference between the groups regarding the type of bacteria and microbial sensitivity (Table 4).

3.3. Patients’ data base interactions with microbiological results

Most patients with urinary positive bacterial growth were classified as SB due to absent clinical signs (Table 2). Dogs with HC were classified as showing SB in 9/74 (12.2%), while the SBC cases (3/74 – 4.1%) were all in the WCHC group. In contrast, control group showed SB in 5/19 (26.3%) and SBC in 4/19 (21.1%) of cases. There was an association between the SBC clinical manifestation and HC absence (Control group), as well as with the degree of HC control (P = 0.021). Considering ultrasound and laboratory findings, all dogs with positive cultures were considered as with lower urinary tract infection, since no pyelonephritis was observed.

Among controls, 4/19 dogs (21.1%) showed clinical signs compatible with UTI (stranguria, n = 1; urinary incontinence, n = 1; pollakiuria and hematuria, n = 1; and urinary incontinence and hematuria, n = 1). Remaining patients included as controls were suspect of UTI because of compatible abnormalities in urine sediment (Fig. 1), and/or due thickening and irregularity of bladder wall, observed in 10/19 dogs (52.6%)
in ultrasonographic examination. One of the dogs with hematuria at urinalysis had a negative microbiological culture and the presence of prostatic cysts on the ultrasound, suggesting non-bacterial prostatitis and/or prostatic hyperplasia.

Only four dogs in the WCHC group showed compatible UTI clinical signs (hematuria, \( n = 1 \); urinary incontinence, \( n = 1 \); pollakiuria, \( n = 1 \); and reported putrid smell in the urine by the owner, \( n = 1 \)). From these dogs, three presented bladder walls thickening at ultrasound and only them showed positive urine culture. The patient with urinary incontinence had negative urine culture result. None of the patients in the PCHC group presented US, clinical signs, or alterations compatible with UTI; however, two had a positive result in culture and were considered as SB. The four patients in the NDHC group with positive culture showed ultrasound changes that suggested SBC (thickened and irregular bladder wall, and/or the presence of hyperechoic sediment), although no SBC history.

Regarding leukogram (Table 5), there was no difference in the total leukocyte count (\( P = 0.682 \)) and neutrophils (\( P = 0.373 \)) between all groups. However, when comparing all groups of HC patients with the Control group, lymphopenia was more evident in patients with HC (\( P = 0.041 \)). There was association (\( P = 0.005 \)) between positive culture in ultrasonographic examination. One of the dogs with hematuria at urinalysis had a negative microbiological culture and the presence of prostatic cysts on the ultrasound, suggesting non-bacterial prostatitis and/or prostatic hyperplasia.

Only four dogs in the WCHC group showed compatible UTI clinical signs (hematuria, \( n = 1 \); urinary incontinence, \( n = 1 \); pollakiuria, \( n = 1 \); and reported putrid smell in the urine by the owner, \( n = 1 \)). From these dogs, three presented bladder walls thickening at ultrasound and only them showed positive urine culture. The patient with urinary incontinence had negative urine culture result. None of the patients in the PCHC group presented US, clinical signs, or alterations compatible with UTI; however, two had a positive result in culture and were considered as SB. The four patients in the NDHC group with positive culture showed ultrasound changes that suggested SBC (thickened and irregular bladder wall, and/or the presence of hyperechoic sediment), although no SBC history.

Regarding leukogram (Table 5), there was no difference in the total leukocyte count (\( P = 0.682 \)) and neutrophils (\( P = 0.373 \)) between all groups. However, when comparing all groups of HC patients with the Control group, lymphopenia was more evident in patients with HC (\( P = 0.041 \)). There was association (\( P = 0.005 \)) between positive culture

### Table 3

<table>
<thead>
<tr>
<th>NDHC (n = 4)</th>
<th>PCHC (n = 2)</th>
<th>WCHC (n = 6)</th>
<th>CONTROL (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram –</td>
<td>Escherichia coli (25%), Cireobacter sedlakii (25%), Klebsiella variicola (25%)</td>
<td>Escherichia coli (16.6%), Enterobacter aerogenes (16.6%)</td>
<td>Escherichia coli (44.4%), Proteus mirabilis (11.1%)</td>
</tr>
<tr>
<td>Gram +</td>
<td>Staphylococcus pseudintermedius (25%)</td>
<td>–</td>
<td>Staphylococcus capit (16.6%), Bacillus firmus (16.6%), Arthrobacter gandavensis (16.6%), Corynebacterium cellulare (16.6%), Corynebacterium cellulosum (11.1%), Enterococcus sp. (11.1%)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>–</td>
<td>–</td>
<td>One bacterium missed in MALDI-TOF (11.1%)</td>
</tr>
</tbody>
</table>

NDHC, newly diagnosed hypercortisolism; PCHC, poorly controlled hypercortisolism; WCHC, well controlled hypercortisolism.

### Table 4

| Bacterial groups (Gram positives or negatives) in positive cultures in each group, persistent infection cases, and antimicrobials sensibility. |
|---------------------|---------------------|---------------------|---------------------|---------------------|
| NDHC n = 4 (%)   | PCHC n = 2 (%)   | WCHC n = 6 (%)   | CONTROL n = 8 (%)   | P     |
| Gram –                         | Gram +                         | Persistent infection | ATM sensitivity |               |
| Escherichia coli (75)       | –                             | –                     | –                     | –               |
| Gram +                         | –                             | –                     | –                     | –               |
| Persistent infection          | –                             | –                     | –                     | –               |
| AMC 2 (50)                  | 2 (100)                      | 4 (66.7)               | 7 (77.8)               | 1.000 |
| CFE 1 (25)                  | 1 (50)                       | 4 (66.7)               | 6 (66.7)               | 0.458 |
| DOX 1 (25)                  | 2 (100)                      | 3 (50)                 | 3 (33.3)               | 0.254 |
| NIT 2 (50)                  | 2 (100)                      | 3 (50)                 | 5 (55.6)               | 0.722 |
| SUT 4 (100)                 | 1 (50)                       | 5 (83.3)               | 6 (66.7)               | 0.545 |
| ENR 3 (75)                  | 0 (0)                        | 4 (66.7)               | 7 (77.8)               | 0.116 |

NDHC, newly diagnosed hypercortisolism; PCHC, poorly controlled hypercortisolism; WCHC, well controlled hypercortisolism; ATM, antimicrobial; AMC, amoxicillin with clavulanic acid; CFE, cephalaxin; DOX, doxycycline; NIT, nitrofurantoin; SUT, sulfamethazole with trimethoprim; ENR, enrofloxacin.

**Fig. 1.** Frequency of urinary sediment abnormalities and proteinuria in each group. Vertical axis shows urinary sediment quantification in cells per field (leukocyturia, cytologically evident bacteriuria, and hematuria) by optic microscopy (400×) and proteinuria quantification in milligrams per deciliter by dipstick. The horizontal axis shows the frequency (%) of each change by group.
and lymphocyte count below 1500 cells/μL in patients with HC (Fig. 2). Table 5 shows hematologic, serum biochemistry, and ACTH stimulation tests’ results from patients in the study.

Eleven out 21 dogs (57.9%) with positive bacterial growth showed leukocyturia or pyuria and 16/21 (84.2%) dogs presented variable degrees of cytologically evident bacteriuria at urinalysis. The frequency of changes in urine sediment findings in the distinct groups is shown in Fig. 1. A certain independence can be observed between positive results in urine culture and changes in urinary sediment, since there was a considerably larger number of samples with sediment changes suggesting infection; however, without bacterial growth. Despite urine sediment examination findings were not able to predict positive bacterial growth in dogs with HC or in controls, there was association ($P = 0.005$) between the positive cultures and USG below to 1020 in patients with HC (Fig. 3). Patients from control and WCHC groups showed more pronounced leukocyturia in comparison with dogs from NDHC and PCHC groups ($P = 0.034$).

### 3.4. Follow-up after antimicrobial treatments

From the 21 patients exposed to antimicrobial treatment based in their respective bacterial susceptibility, microbiological cure was not reached in four (19%). These were cases associated with *E. coli* infections and could be classified as SB before treatment since no clinical signals were documented; except by one dog whose owner reported persistent putrid smell in the dog’s urine. This particular patient was the only from WCHC with persistent infection. Other two cases occurred in PCHC group, and another one was a control dog. Persistent infection rates by group are shown in Table 4.

*E. coli* was isolated in follow-up urine culture from all persistent cases. Despite resistances to more antimicrobials were documented, cases still were considered SB (no clinical signs). However, the putrid urine smell from refractory WCHC group’s dog only resolved after microbiological cure at the third antimicrobial cycle with different antibiotic. Despite this particular patient have always been considered as well-controlled HC due to its overall clinical and laboratory results features, refractory UTI and chronic lymphopenia motivated a 20% trilostane dose increment implemented along the last antibiotic cycle. The other three refractory cases reached microbiological cure after the second cycle with antibiotic modifying.

### Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NDHC (n = 27)</th>
<th>PCHC (n = 21)</th>
<th>WCHC (n = 34)</th>
<th>CONTROL (n = 19)</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count</td>
<td>11.6 ± 6.2</td>
<td>10.8 ± 3.1</td>
<td>8.9 ± 2.8</td>
<td>13.8 ± 6.3</td>
<td>6–17 (x10³/μL)</td>
</tr>
<tr>
<td>Seg. neutrophils</td>
<td>8.96 ± 5.7</td>
<td>8.49 ± 2.7</td>
<td>7.01 ± 2.2</td>
<td>9.6 ± 4.9</td>
<td>3–11.5 (x10³/μL)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.4 ± 0.7</td>
<td>1.2 ± 0.5</td>
<td>1.3 ± 0.6</td>
<td>1.9 ± 1</td>
<td>1–4.8 (x10³/μL)</td>
</tr>
<tr>
<td>USG</td>
<td>1020 ± 102</td>
<td>1026 ± 1,017</td>
<td>1026 ± 1,013</td>
<td>1040 ± 1,015</td>
<td>&gt;1,030</td>
</tr>
<tr>
<td>ALP</td>
<td>852 ± 1,259</td>
<td>531.1 ± 612.3</td>
<td>422.9 ± 714.3</td>
<td>134 ± 141.9</td>
<td>&lt;156 (U/L)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.83 ± 0.35</td>
<td>0.81 ± 0.35</td>
<td>0.66 ± 0.22</td>
<td>0.95 ± 0.35</td>
<td>0.5–1.5 (mg/dL)</td>
</tr>
<tr>
<td>Urea</td>
<td>48.4 ± 22.9</td>
<td>48.7 ± 21.2</td>
<td>48.7 ± 20.8</td>
<td>45.2 ± 17.6</td>
<td>21–60 (mg/dL)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>343.3 ± 203.7</td>
<td>280.6 ± 151.7</td>
<td>226 ± 87.3</td>
<td>193.8 ± 5.26</td>
<td>135–270 (mg/dL)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>233.9 ± 234.9</td>
<td>182.9 ± 138.8</td>
<td>228.6 ± 217.4</td>
<td>133.3 ± 134.7</td>
<td>20–112 (mg/dL)</td>
</tr>
<tr>
<td>Glucose</td>
<td>106.6 ± 21.3</td>
<td>107.5 ± 22.7</td>
<td>112.5 ± 26.6</td>
<td>95.1 ± 24.8</td>
<td>65–118 (mg/dL)</td>
</tr>
<tr>
<td>Cortisol (1h-AS)</td>
<td>23.12 ± 11.5</td>
<td>9.55 ± 3.7</td>
<td>4.54 ± 1.5</td>
<td>–</td>
<td>6–17 (μg/dL)</td>
</tr>
</tbody>
</table>

NDHC, newly diagnosed hypercortisolism; PCHC, poorly controlled hypercortisolism; WCHC, well controlled hypercortisolism; WBC, white blood cell; USG, urine specific gravity; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AS, after ACTH stimulation.

---

**Fig. 2.** Lymphocytes count (x10³/μL) distribution accordingly with absent bacterial growth in 48 h (ABG 48h) or positive growth in the samples of dogs with hypercortisolism and in control dogs.
Comparative Immunology, Microbiology and Infectious Diseases 75 (2021) 101624

6

antimicrobial treatment was generally very well tolerated and no re-

observed that this management was clearly associated to increased

cases documented as stated by current ISCAID guideline [9]. In contrast,

the previous ISCAID guidelines published in 2011 [11]. However, we

UTI, adverse drug reactions, increased costs, and higher risk of re-
in most compromised patients is associated with short-term increase of

bacteriuric cases [15], this lower frequency could be also related to

infections along the follow up period were documented.

The arguments for these recommendations included the fact that anti-

ment of bacterial urinary tract infections in dogs and cats do not

recommend SB antimicrobial treatment regardless of endocrine comorbidities [9]. This new recommendation published in 2019 push veterinarians to review their concepts regarding UTI in dogs with HC. The arguments for these recommendations included the fact that antibiotic treatment of human patients with asymptomatic bacteriuria even in most compromised patients is associated with short-term increase of UTI, adverse drug reactions, increased costs, and higher risk of re-infections with antimicrobial-resistant pathogens [9,15]. Despite these arguments were never been adequately tested in dogs with HC, current ISCAID guidelines consider there is no evidence, or apparent reason to suspect, that the situation would be different in dogs with comorbidities such as HC [9]. Nevertheless, our results can partially support these recommendations. The decision to treat all dogs with positive bacterial growth in cultures regardless of clinical signs absence was supported by the previous ISCAID guidelines published in 2011 [11]. However, we observed that this management was clearly associated to increased owners’ costs and selection of more resistant bacteria in the refractory cases documented as stated by current ISCAID guideline [9]. In contrast, antimicrobial treatment was generally very well tolerated and no re-infections along the follow up period were documented.

Our study shows a lower frequency of UTI in urine samples from dogs with HC (14.6%) when compared to previous studies that demonstrated frequencies between 39 and 50% [5–7]. However, this smaller bacteriuria prevalence agrees with a recent study [15] which showed a 18% UTI prevalence; being the majority classified as SB. Despite the argument that dogs with HC included could have been previously treated with antimicrobials in the past, reducing the overall prevalence of bacteriuric cases [15], this lower frequency could be also related to current quicker HC suspicion and identification when compared to the past [14]. This key factor reduces the time of exposition to GC immuno-suppressive effects [1].

The fact that dogs with HC often have SB characterized only by a positive urine bacterial culture, but without clinical manifestations [9] needs attention due to the increased risk of complications such as pyelonephritis. However, this complication does not seem common in dogs with HC [14,15], and prospective studies evaluating the chronic impact of untreated SB are lacking [9,15]. Moreover, there is no evidence of an association between SB and risk of development of cystitis or other infections in dogs, cats, and humans [9].

Only three dogs from the WCHC group out 74 patients (4.1%) with HC included had SBC. This prevalence is in accordance with previous studies that suggested that less than 5% of dogs with HC and UTI will present clinical signs [5,7], and that clinical presentation of UTI is quite variable in dogs [21]. Also, typical clinical signs of bacterial cystitis are potentially suppressed by HC [1,14] making SBC a more probable diagnosis for well controlled dogs than newly diagnosed or poorly controlled dogs. By this way, SBC and SB may be misclassified in HC.

Newly diagnosed dogs with HC have had a lower occurrence of urine positive cultures, and probably, HC early diagnosis was essential for this finding in our study. Host innate immunity processes, characterized by the release of inflammatory mediators and cytokines, as well as inflammation-related serum phagocytes and proteins [22,23], can be inhibited by cortisol, predisposing to opportunistic infection. Instead, due to a marked inflammatory response, there may be a serious lesion in the lower urinary tract mucosa, predisposing to the perpetuation and chronification of the infectious and inflammatory condition [24,25]. A study revealed that immunocompromised rats, which cannot build an adequate lymphocyte response in the lower urinary tract, are also resistant to the development of chronic cystitis [26]. In patients with HC this type of acute response is impaired [1] and, therefore, could prevent the maintenance of the epithelium colonization process by microorganisms, which may explain the lower prevalence of SBC. These immunity failures in the patient with HC may explain the difference in the prevalence of UTI between the control group and the PCHC group.

Dogs with HC and low grade leukocyturia presented SB, which can be defined as bacteriuria determined by positive bacterial culture in the absence of clinical signs related to UTI [9]. The inability of the immune system to organize an inflammatory response in the bladder epithelium under the effect of GC can cause lower leukocyte migration [5,7]. Several studies reported that SB is common in healthy dogs, being present in 2.1–12% of cases [27–31]. Other suggest a higher prevalence (15–74%) in dogs with metabolic diseases such as diabetes mellitus.

Fig. 3. Urine specific gravity distribution accordingly with absent bacterial growth in 48 h (ABG 48 h) or positive growth in the samples of dogs with hyper-cortisolism and in control dogs.

4. Discussion

As preconized for humans, current ISCAID guidelines for management of bacterial urinary tract infections in dogs and cats do not recommend SB antimicrobial treatment regardless of endocrine comorbidities [9].
E. coli which they took part. Also, all persistent infection cases were caused by specificity technique for microorganism identification in urine [20]. MALDI-TOF technology is considered an extremely high sensitivity and bacteria prevalence when compared to Gram-positive bacteria in the study. Cystitis should be avoided. Expose such a dog to antibiotic treatment may be associated to unnecessary costs, clinical risks (e.g. cystoentesis, collateral effects), and MDR-microorganisms selection.

5. Conclusions

This research showed a reduction on the UTI frequency in canine population with HC when compared to previous studies, being most cases classified as SB. However, current concepts of SB and SBC may not be perfect adequate to dogs with HC since the disease may suppress clinical signs of cystitis, and the risk for major consequences of do not treat SB in these dogs is unknown. In contrast, SBC diagnosis is more probable in patients with well-controlled HC. Additionally, dogs with HC and lymphopenia and/or low USG, as well as those with poorly controlled HC, are more likely to develop UTI. Since the majority of UTI in dogs with HC are SB, the indication for performing urine culture as part of the routine exams in a dog with HC without clinical signs of cystitis should be avoided.

Acknowledgements

The authors would like to thank DVM., MSc. Guilherme Luiz Carvalho de Moura Martins for their valuable contributions towards this project concept. Also, we would like to thank to the entire staff of the Imaging Service of the Hospital de Clínicas Veterinárias from the Universidade Federal do Rio Grande do Sul for their help with ultrasound evaluations and urine sampling.

References


Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

The authors would like to thank DVM., MSc. Guilherme Luiz Carvalho de Carvalho and DVM. MSc. Francisco Sávio de Moura Martins for their valuable contributions towards this project concept. Also, we would like to thank to the entire staff of the Imaging Service of the Hospital de Clínicas Veterinárias from the Universidade Federal do Rio Grande do Sul for their help with ultrasound evaluations and urine sampling.