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Urinary tract infections in dogs with spontaneous hypercortisolism – frequency, symptoms and involved pathogens

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Vetsuisse-Faculty University of Zurich (2020)

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Urinary tract infections in dogs with spontaneous hypercortisolism - frequency, symptoms and involved pathogens

Dogs with hypercortisolism (HC) are predisposed to developing bacteriuria associated either with clinical signs of cystitis or without clinical signs (subclinical bacteriuria). Based on current guidelines, dogs with subclinical bacteriuria should not be treated with antibiotics. Before these guidelines were published in 2019, dogs with HC and bacteriuria were commonly treated with antibiotics. The aims of this study were to investigate dogs with HC for the presence of bacterial cystitis and subclinical bacteriuria, to address the pathogens involved, and to assess the outcome of antibiotic treatment. Dogs newly diagnosed with HC between 2005 and 2015 from which a urine bacterial culture was available were included. Of the 161 client-owned dogs included, 29 showed bacteriuria, which was subclinical in 24 cases. *Escherichia coli* was the most commonly isolated pathogen. In 14 dogs, follow-up data was available, of which 13 were treated with antimicrobial. Follow-up bacterial culture was negative in 10 treated dogs. Bacteriuria persisted in three treated dogs and the one untreated dog. The prevalence of positive bacterial urinary culture in dogs with HC was lower than previously reported. In the majority of dogs, bacteriuria was subclinical. Most dogs had a negative bacterial culture result after antimicrobial treatment; however, more resistant bacteria were detected in persistently positive urine.

Keywords:

Antimicrobial resistance, canine, hypercortisolism, subclinical bacteriuria, urine culture

Vetsuisse-Fakultät Universität Zürich (2020)

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Harnwegsinfektionen bei Hunden mit Hyperkortisolismus - Häufigkeit, Symptome und beteiligte Krankheitserreger

Hunde mit Hyperkortisolismus (HC) neigen dazu eine Bakteriurie zu entwickeln, mit oder ohne klinische Anzeichen einer Blasenentzündung. Nach den aktuellen Behandlungsempfehlungen sollten Hunde mit subklinischer Bakteriurie nicht mit Antibiotika therapiert werden. Vor der Veröffentlichung dieser Richtlinien, wurden Hunde mit HC und Bakteriurie meist mit Antibiotika behandelt. Ziel dieser Studie war, Hunde mit HC auf das Vorhandensein einer bakteriellen Blasenentzündung und subklinischen Bakteriurie zu untersuchen, die beteiligten Erreger zu identifizieren und den Therapieerfolg einer Antibiotikabehandlung zu bewerten. Verwendet wurden 161 Hunde mit HC (diagnostiziert zwischen 2005-2015) und einer verfügbaren Urin-Bakterienkultur. Bei 29 Hunden wurde eine Bakteriurie diagnostiziert, die in 24 Fällen subklinisch war. *Escherichia coli* war der am häufigsten isolierte Erreger. Bei 14 Hunden (13 mit Antibiotika behandelt) lagen Daten einer Nachuntersuchung vor. Die Bakterienkultur war bei 10 der behandelten Hunde negativ. Eine persistierende Bakteriurie wurde bei drei therapierten Hunden und einem unbehandelten Hund festgestellt. Die Prävalenz einer positiven bakteriellen Harnkultur bei Hunden mit HC war tiefer als bisher publiziert. Bei der Mehrzahl der Hunde war die Bakteriurie subklinisch. Die meisten Hunde hatten nach der antimikrobiellen Therapie eine negative Bakterienkultur; doch bei persistierenden positiven Urinkulturen wurden Antibiotika resistentere Keime nachgewiesen.

Stichworte:

Antibiotikaresistenz, Hund, Hyperkortisolismus, subklinische Bakteriurie, Urinkultur

2. Manuskript

Urinary tract infections in dogs with spontaneous hypercortisolism – frequency, symptoms and involved pathogens

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2.1 Abstract

Dogs with hypercortisolism are predisposed to developing bacteriuria associated either with clinical signs of cystitis or without clinical signs (subclinical bacteriuria). Based on current guidelines, dogs with subclinical bacteriuria should not be treated with antibiotics because there is no evidence that treatment improves outcome and because unnecessary treatments should be avoided. Before these guidelines were published in 2019, dogs with HC and bacteriuria were commonly treated with antibiotics irrespective of clinical signs. Comprehensive data on the frequency of bacterial cystitis, subclinical bacteriuria and the outcome of antimicrobial treatment in dogs with hypercortisolism is sparse. The aims of this study were to investigate dogs with hypercortisolism for the presence of bacterial cystitis and subclinical bacteriuria, to address the pathogens involved, and to assess the outcome of antibiotic treatment. Dogs newly diagnosed with hypercortisolism between 2005 and 2015 from which a urine bacterial culture was available were included. Statistical analysis was performed with non-parametric tests. Of the 161 client-owned dogs included, 29 (18%) showed bacteriuria, which was subclinical in 24 (83%) cases. *Escherichia coli* was the most commonly isolated pathogen (58%). Bacteriuria was not associated with sex or neutering status. In 14 dogs, follow-up data was available, of which 13 (93%) were treated with antimicrobials for 14 to 28 days. Follow-up bacterial culture (1 to 118 days after cessation of therapy) was negative in 10 (77%) treated dogs; a negative follow-up culture was not associated with gender, age or duration of treatment. Bacteriuria persisted in three treated dogs and the one untreated dog. The prevalence of positive bacterial urinary culture in dogs with hypercortisolism was lower than previously reported. In the majority of dogs, bacteriuria was subclinical. Most dogs had a negative bacterial culture result after antimicrobial treatment; however, more resistant bacteria were detected in persistently positive urine.

Key words: antimicrobial resistance, canine, hypercortisolism, subclinical bacteriuria, urine culture results

2.2 Introduction

Spontaneous hypercortisolism (HC), one of the most common endocrinopathies in dogs, induces a state of immunosuppression which increases susceptibility to infections.[11,13,15,36] As a result, dogs with HC often develop bacteriuria, comparable to dogs under long-term therapy with glucocorticoids or immunocompromised dogs.[12,16,37,38] According to current veterinary guidelines, the presence of bacteria in the urine determined by a positive bacterial culture, together with clinical signs (pollakisuria, dysuria, periuria, stranguria, hematuria), is defined as sporadic bacterial cystitis. A positive bacterial culture in the absence of clinical signs is defined as subclinical bacteriuria, a fairly common condition with reported rates of 2.1–12% in healthy dogs.[26,33,35,40,41,42] Dogs with comorbidities like endocrinopathies (e.g. diabetes mellitus (DM), HC) or dogs treated with cyclosporine or glucocorticoids are even more frequently affected, with rates of 15-74%.[3,4,12,19,27,35,38]

Nowadays, a positive urinary culture is still a leading reason for the use of antimicrobials in small animal practice.[17] Regarding treatment, the previous guidelines of the International Society for Companion Animal Infectious Diseases (ISCAID) supported treatment of animals with subclinical bacteriuria and comorbidities such as diabetes mellitus or hypercortisolism.[43] The current guidelines, published in 2019, emphasize not treating subclinical bacteriuria independently of comorbidities.[42] This is also in agreement with actual treatment guidelines for humans.[2,7,31] Antibiotic treatment of human patients with asymptomatic bacteriuria is associated with negative effects, such as the short-term increase of urinary tract infections, adverse drug reaction or increased costs.[10,20,28-30] Additionally, a higher risk of reinfection with antimicrobial-resistant organisms has been seen after treatment.[28]

Until recently, most dogs with HC and bacteriuria were treated with antimicrobials, irrespective of the presence of clinical signs. Data on the frequency of bacterial cystitis and subclinical bacteriuria and the outcome of antimicrobial treatment in this patient group is, however, sparse.[16,23] Therefore, the present study aimed to retrospectively evaluate dogs with

HC diagnosed between 2005 and 2015 for the presence of bacterial cystitis and subclinical bacteriuria, to address the pathogens involved, and to evaluate the effect of antimicrobial treatment on follow-up culture results. The scientific hypothesis was that bacteriuria in dogs with HC is often subclinical and that antimicrobial treatment will often not lead to bacterial cure.

2.3 Materials and Methods

Study design

The medical record database of the Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, was retrospectively searched to identify client-owned dogs newly diagnosed with HC between 2005 and 2015. Only dogs with a quantitative bacterial urinary culture at their first presentation were included. From each dog, the following information was recorded: history, signalment, body weight, physical examination findings, results of urine analysis, urine bacterial culture and follow-up urine bacterial culture results. Dogs with HC and concurrent DM were included in the study. Dogs that received immunosuppressive drugs (chemotherapy, azathioprine, cyclosporine, mycophenolate mofetil, leflunomide) or that had been pre-treated with trilostane before first presentation were excluded.

Diagnosis of HC was based on clinical signs suspicious for HC (e.g. polyuria, polydipsia, polyphagia, hair coat and skin changes, pendulous abdomen, muscle wasting), typical laboratory results (e.g. increased alkaline phosphatase activity, hypercholesterolemia, thrombocytosis) and at least one positive screening test (low-dose dexamethasone suppression (LDDS) test and/or ACTH stimulation test)).[5] In all dogs, treatment of the HC was carried out with trilostane (Vetoryl®, MSD Animal Health GmbH, Luzern, Switzerland), a competitive inhibitor of the 3-β-hydroxysteroid-dehydrogenase, as published elsewhere.[8] None of the dogs underwent adrenalectomy.

Dogs with HC were assessed for the presence of bacteriuria, which was defined as a positive urine bacterial culture result (≥ 10^3 cfu/ml) obtained from an aseptically collected urine sample. Dogs with a positive bacterial culture were assessed for clinical signs of cystitis (pollakisuria, dysuria, periuria, stranguria and hematuria) and for the bacterial species identified in culture. Bacterial cystitis was defined as the presence of bacteriuria in a dog with clinical signs compatible with cystitis (see above). Subclinical bacteriuria was defined as the presence of bacteriuria in the absence of clinical signs of cystitis.[42] Pyuria in the absence of clinical signs was not classified as bacterial cystitis.[42] Due to the retrospective nature of this study, sporadic bacterial cystitis could not be differentiated from recurrent bacterial cystitis. Therefore, the term bacterial cystitis was used for both groups of patients.

For dogs with bacteriuria, follow-up information was collected, which included type and duration of antibiotic treatment, results of follow-up bacterial cultures and outcome of treatment (negative or positive follow up bacterial culture result). To evaluate whether treatment regimens, frequencies of bacterial cystitis and subclinical bacteriuria, and antimicrobial resistance patterns of the uropathogens changed over time, data from dogs diagnosed between 2005 and 2010 were analysed in comparison with dogs diagnosed between 2011 and 2015. Furthermore, results of dogs with HC with and without DM were compared.

Urine analysis

Urine collection was aseptically performed by cystocentesis under ultrasonographic guidance. Routine urine analysis was performed at the Clinical Laboratory, Vetsuisse Faculty, University of Zurich and included urine specific gravity (USG), dipstick analysis, and microscopic examination (40X) of urine sediment. Pyuria was defined as > 4-8 white blood cells (WBC) per high-power field.

The urine samples used for bacterial culture were preserved in sterile plain tubes and stored at 5 °C for not more than 24 hours before setting up the culture. Urine bacterial culture analysis was performed at the Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of

Zurich. A result of ≥ 10^3 cfu/mL of urine was considered to indicate bacteriuria.[4] For all positive bacterial cultures, susceptibility testing was performed. The following antimicrobial classes were included: aminopenicillins, potentiated aminopenicillins, 1st - 4th generation cephalosporins (only 1st, 3rd and 4th), aminoglycosides, tetracyclines, sulfonamides and combination preparation (trimethoprim), fluoroquinolones, amphenicols, macrolides, nitrofurans, polymyxins, steroid antibiotics, lincosamides, ansamycins and nitroimidazoles. The *E. coli* isolates were analysed as to their antimicrobial resistance patterns over time; only antimicrobial classes that were tested in all *E. coli* isolates were included in this analysis.

Statistical analysis

All comparisons were conducted using the non-parametric Chi square and Fisher's exact test (for expected frequencies < 5) and the Mann-Whitney test for categorical variables. A $p < 0.05$ was considered significant. All statistical analyses were performed using the IBM SPSS 22.0 software package.

2.4 Results

Animals

One hundred and sixty-one client-owned dogs with newly diagnosed HC between 2005 and 2015 were enrolled in the study. At the time of diagnosis, age ranged from 3 to 16 years (median 11) and body weight from 2.3 to 61 kg (median 12.2). Eighty-eight dogs (55%) were female (71 spayed) and 73 (45%) were male (40 castrated). In total, 58 different breeds were recorded. The most frequently represented breeds were mixed breed dogs (n=35), followed by Yorkshire Terriers and Dachshunds (of each breed n=11), West Highland White Terriers (n=10) and Poodles (n=7). Seventeen (11%) dogs had concurrent DM and were treated with different types of insulin.

In 49 (30%) dogs, there were indications in the history of pre-treatment with antibiotics before the first presentation. There was no significant association of antimicrobial pre-treatment with a positive urinary culture result (p=0.736).

Bacterial culture and microbial inhibition test results

A positive bacterial culture was detected in 29 (18%) of the dogs at first presentation. Nineteen of these dogs (66%) were females (16 spayed) and 10 (34%) were males (four castrated). There was no significant association of bacteriuria with sex and neutering status of the dogs (sex, p=0.195; neutering status, p=0.312).

A microbial inhibition test to detect antibiotic residues in the sample was routinely performed as part of the urine bacterial culture. Results are shown in Table 1. Nine of the 29 dogs (31%) with a positive culture result and 40 of the 132 dogs (30%) with a negative culture result had a history of pre-treatment with antimicrobials.

Clinical signs and urine sediment analysis

Five dogs (17%) with positive culture results showed clinical signs consistent with cystitis (stranguria, n=2; pollakisuria, n=1; hematuria, n=1; periuria, n=1). The remaining 24 dogs (83%) showed subclinical bacteriuria. Of the 132 dogs with negative culture results, nine (7%) showed clinical signs consistent with cystitis (stranguria, n=0; pollakisuria, n=2; hematuria, n=2; periuria, n=5).

Microscopic examination of the urine sediment was available from 24 dogs with positive bacterial culture results and from 124 dogs with negative bacterial culture results.

In the 24 dogs with positive bacterial culture results, bacteria in the sediment in combination with pyuria occurred in 15 (63%) dogs. Two of these dogs had clinical cystitis and 13 dogs subclinical bacteriuria. Pyuria in the absence of visible bacteria was present in two dogs (8%), which both had clinical cystitis. Visible bacteria but no pyuria occurred in two dogs (8%); both had subclinical bacteriuria. Five dogs (21%) had no bacteria and normal urine sediment analyses; no dogs showed clinical signs of cystitis.

In the 124 dogs with negative bacterial culture results, bacteria in the sediment in combination with pyuria occurred in 1 (0.8%) dogs; this dog showed no clinical signs of cystitis. Pyuria in the absence of visible bacteria was present in nine dogs (7.2%); eight of these dogs had no clinical signs of cystitis and one dog showed signs of cystitis. Visible bacteria but no pyuria occurred in two dogs (1.6%); both had no clinical signs of cystitis. A total of 112 (90%) had no bacteria and a normal urine sediment analysis; eight of these dogs showed clinical signs of cystitis.

Isolated pathogens

A total of 33 pathogens were identified in the 29 dogs with a positive bacterial culture (Figure 1). *E. coli* (n= 19, 58%) was the most common uropathogen. Twenty-six dogs (90%) showed monoinfections, while three dogs (10%) showed mixed infections (*E. coli* and *Klebsiella* spp., n=1; *E. coli* and *Proteus* spp., n=1; *E. coli* and *Proteus* spp. and *Enterococcus* spp., n=1). Fourteen (42%) isolates were susceptible to aminopenicillins, which are regarded as the first-line empirical treatment for bacterial cystitis in dogs in Switzerland.[1,34,42] A total of 22 (67%) of the isolates were susceptible to potentiated aminopenicillins, 21 (64%) to sulfonamides and combinations (trimethoprim) and 20 (61%) to fluoroquinolones.

The antimicrobial resistance patterns of the 19 *E. coli* isolates are shown in Figure 2, separated for the year of isolation. Nine (47%) *E. coli* isolates were susceptible to aminopenicillins, 12 (63%) to potentiated aminopenicillins, 12 (63%) to sulfonamides and combinations (trimethoprim) and 13 (68%) to fluoroquinolones.

Follow-up information

A follow-up bacterial culture was available for 14 of the 29 dogs with an initially positive urine culture. Between the two cultures, 13 (93%) dogs had been treated with antibiotics. The time between cessation of antibiotic treatment until the follow-up culture ranged from 1–118 days (median 14 days).

Of the 13 treated dogs, 10 (77%) had a negative follow-up culture result (Table 2). Three dogs

(23%) had a positive follow-up culture result (Table 3); in two dogs, *E. coli* was again detected, which showed resistances to more antimicrobial classes than before treatment. The dog that had not undergone antimicrobial treatment also showed a positive bacterial culture result 63 days after first examination; there was no increase in antimicrobial resistance of the *E. coli* and *Klebsiella* spp. in the second culture of this dog.

There was no significant difference in sex, age and duration of treatment between dogs that had a negative or positive bacterial culture result after antimicrobial treatment (sex, p=0.689; age, p=0.839; duration of treatment, p=0.937).

Assessment of the study population over time

Seventy-five of the 161 dogs with HC were diagnosed between 2005-2010 (group 1) and 86 of 161 between 2011-2015 (group 2). There was no difference in the frequency of bacterial cystitis and subclinical bacteriuria between the two time periods (p=0.632). The number of resistances of the *E.coli* pathogens to the antimicrobial classes tested did not differ between the two groups either (Figure 2) (p=0.319).

Dogs with concurrent diabetes mellitus

Seventeen of the 161 dogs (11%) had concurrent DM, of which five (29%) had a positive urine bacterial culture result. There was no difference regarding the prevalence of bacterial cystitis and subclinical bacteriuria in dogs with HC with or without concurrent DM (p=0.196).

2.5 Discussion

In the present study, 18% of the dogs with spontaneous HC showed bacteriuria. The prevalence of bacteriuria was similar to that in recently published studies with prevalences of 24% and 18%, but lower than in older studies, where 46% and 50% were reported.[6,9,12,23] Since the methods used for urine sample collection and diagnosis varied markedly between the studies, a direct comparison of these prevalences is difficult. Bacteriuria was associated with neither gender nor age of the dogs in our study, which is at odds with previous studies, where female dogs were predisposed to show bacteriuria.[18,22,37] Also, the neutering status of the dogs had no effect on the prevalence of positive bacterial urinary culture. A possible reason for these conflicting findings may be that the dog population in the present study was too small to reach the level of significance.

A total of 83% of the cases with a positive urinary culture in our study showed subclinical bacteriuria, which represented 15% of all investigated dogs with HC. This is higher than reported for healthy dogs (2.1–12%).[26,33,35,40,41] The percentage of dogs with clinical signs of cystitis in our study is relatively high (17% of dogs with bacteriuria, 3% of all dogs with HC) compared to the results of the work of Forrester et al., where < 5% of the dogs with bacteriuria showed lower urinary tract signs.[12] The higher percentage of bacterial cystitis within our study could be due to the very detailed history (including specific questions regarding typical symptoms of cystitis) taken in every dog with HC, which is standard procedure at our clinic. Nonetheless, due to the retrospective nature of this study, it cannot be excluded that some signs of HC such as polyuria were misinterpreted as signs of bacterial cystitis (e.g. periuria or pollakisuria) by the clinicians. This could also explain the finding that eight dogs with clinical signs of cystitis had a negative urine culture result and a normal sediment analysis.

In small animal practice, bacterial urinary tract infections are a leading reason for the use of antibiotics.[17] The current guidelines emphasize not treating subclinical bacteriuria (often independently of comorbidities like HC) with antimicrobials, but this represents a change from the previous guidelines.[42,43] The latter still supported antimicrobial treatment of animals with subclinical bacteriuria and comorbidities such as hypercortisolism, as a positive urinary culture in these cases was classified as a “complicated urinary tract infection” with the fear of ascending infections due to immunocompromised status. Accordingly, 13 of 14 dogs with HC, bacteriuria and follow-up information investigated in this study were treated with antimicrobials. In 10 of 14 (77%) dogs the follow-up bacterial culture was negative. Due to the retrospective nature of this study, the time until the second culture was performed was not standardized and varied between cases. Furthermore, no standardized repetitive testing over subsequent months was performed. Therefore, recurrence of bacteriuria in these dogs cannot be excluded. Interestingly, treated dogs that had a positive follow-up culture (3 dogs in this study) showed a tendency towards more resistant uropathogens than before treatment, underlining that treatment could have a negative impact.

Urine bacterial culture from aseptically collected urine samples was used to diagnose bacteriuria, which is considered the gold standard for diagnosis.[4] Microscopic evaluation of urine sediment revealed bacteria in only 17 of 24 cases with positive bacterial culture (respectively, in 2 of 4 cases with clinical signs of cystitis and positive bacterial culture), underlining that bacterial culture should always be performed if bacterial cystitis is suspected.

The most frequently isolated uropathogen in this study was *E. coli* (58%), which is consistent with previous studies.[12,16,18,21,32,37] Non-potentiated aminopenicillins are also regarded as first-line empiric treatment in dogs with bacterial cystitis in the national guidelines of Switzerland.[1,34,42] However, only 42% of all isolates and 47% of the *E. coli* isolates in this study were found susceptible to this antibiotic class. Potentiated aminopenicillins were clearly superior, with 67% of all isolates and 63% of the *E. coli* isolates showing susceptibility. Because of the availability of products, potentiated aminopenicillins are most commonly prescribed in dogs with cystitis in Switzerland.[24] Trimethoprim/sulfamethoxazole (TMS), another first-line treatment option, showed comparable susceptibility rates, with 64% of all isolates and 63% of the *E. coli* isolates showing susceptibility. Many clinicians however are reluctant to use TMS in dogs due to potential adverse effects such as idiosyncratic toxicity with possible fever, polyarthryopathy, skin eruptions, hepatotoxicity and keratoconjunctivitis sicca among other symptoms.[39] Interestingly, fluoroquinolones were not clearly superior compared to potentiated aminopenicillins and TMS in this study. Fluoroquinolones are regarded as highest-priority, critically important antimicrobials and should therefore be avoided if possible.

Overall, the frequency of antimicrobial resistances in the *E. coli* in this study was higher than that reported for Switzerland in 2012-2013.[25] In the latter study, 10.5% of the isolates showed resistance to potentiated aminopenicillins, and 13.6% to fluoroquinolones and TMS. In the present studies, 32–37% of the *E. coli* isolates showed resistance to these groups of antimicrobials. This finding could be due to differences in the study population. Dogs with HC might be more exposed to antimicrobials or hospitalization due to comorbidities and age compared to the general dog population; both factors are known to increase the risk for antimicrobial resistant pathogens.[14,44] Furthermore, our samples all originated from dogs presented at an university clinic; many of these patients (30%) were known to be pre-treated with antimicrobials (a point unknown in the study of Marques et al. 2016), which could have had an impact on the development of antimicrobial resistances. Furthermore, variations in the methodology might explain some of these differences.

Our study has some limitations. First, cases were investigated retrospectively, which omitted a long-term follow-up of the cases with repetitive bacterial culture. Furthermore, the allocation of the cases to bacterial cystitis and subclinical bacteriuria was based on the data of the case histories, which might have been incomplete and influenced by the judgment of the clinicians. Furthermore, classification into sporadic bacterial cystitis and recurrent bacterial cystitis was not possible due to the retrospective nature of the study; evaluation of pre-treatment was also incomplete. The inclusion of cases previously treated with antimicrobials may on the one hand have reduced the overall prevalence of bacteriuric cases (clinical and subclinical) at presentation but on the other hand may have increased the prevalence of resistant strains. Finally, despite the large number of dogs with spontaneous HC included in this study, only a relatively small number of patients had a positive bacterial culture result; thus the power might have been too low to reach the significance level for some variables.

Conclusion

In summary, the prevalence of positive bacterial urinary culture in dogs with HC was 18%. Subclinical bacteriuria was much more common (15%) than bacterial cystitis (3%). *E. coli* was the most common pathogen, and resistance to non-potentiated aminopenicillins, and to a lesser extent to potentiated aminopenicillins, TMS and fluoroquinolones, was common. Although 10 of 13 treated dogs showed a negative follow-up culture result, an increase in antimicrobial resistances was observed in two treated dogs with persistent bacteriuria. Future studies should address whether an adequate control of the HC could promote elimination of clinical and subclinical bacteriuria in dogs with spontaneous HC. According to current guidelines, subclinical bacteriuria in dogs with HC should not be treated with antimicrobials.

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2.7 Tables

Table 1*:* Results of the microbial inhibition test to detect antibiotic residues in urine samples. Numbers represent the number of urine samples.

Urine bacterial culture result positive	negative	**Microbiologic inhibitor substance test result** positive	negative	**Known antimicrobial pre-treatment**	**No antimicrobial pre-treatment**	**No data about a possible antimicrobial pre-treatment available**
29		5 (17%)		1	4	
			24 (83%)	8	12	4
	132	45 (34%)		23	19	3
			87 (66%)	17	64	6

Table 2*:* Signalement, type of bacteria, results of antimicrobial susceptibility testing (in the 1st urinary culture) and antibiotic treatment of the ten dogs with negative follow-up culture.

Dog (year of diagnosis)	Age	Gender	Bacteria (1st culture)	Resistances against antimicrobial classes	Antibiotic therapy, type and dose	Antibiotic therapy, duration	Time to 2nd culture after cessation of antimicrobial therapy	2nd culture	Further cultures
Dog 5 (2005)	9y	M	*Escherichia coli*	Aminopenicillins Oxacillin Amphenicols Macrolides	Amoxicillin/ Clavulanic acid 22 mg/kg q12h	14 days	28 days	negative	3rd culture 197 d after cessation of antimicrobial therapy (aftx): positive (*Escherichia coli),* therapy with Enrofloxacin 14d, 4th culture 28d aftx: negative
Dog 6 (2005)	10y	F spayed	*Escherichia coli*	Aminopenicillins Oxacillin Macrolides	Amoxicillin/ Clavulanic acid 19 mg/kg q12h	14 days	9 days	negative	NA
Dog 7 (2005)	10y	F	*Klebsiella oxytoca*	Aminopenicillins Oxacillin Macrolides Nitrofurans	Amoxicillin/ Clavulanic acid 17 mg/kg q12h	14 days	14 days	negative	NA
Dog 8 (2008)	10y	F spayed	*Arcanobacterium species*	Aminopenicillins Oxacillin 1st- 4th generation cephalosporins Sulfonamides and combination preparation (trimethoprim) Fluoroquinolones Amphenicols Macrolides Polymyxins Steroid antibiotics Ansamycins Nitroimidazoles	Amoxicillin/ Clavulanic acid 20 mg/kg q12h	14 days	59 days	negative	NA

Dog 9 (2013)	11y	F spayed	*Escherichia coli*	Aminopenicillins Potentiated aminopenicillins Pirepacillin 1st- 4th generation cephalosporins Tetracyclines Sulfonamides and combination preparation (trimethoprim) Polymyxins	Enrofloxacin 10 mg/kg q24h	21 days	12 days	negative	
Dog 10 (2013)	9y	F spayed	Coagulase-negative *Staphylococcus*	Aminopenicillins Tetracyclines	Amoxicillin/ Clavulanic acid 24 mg/kg q12h	21 days	14 days	negative	NA
Dog 11 (2014)	13y	F spayed	*Escherichia coli*	Aminopenicillins Potentiated aminopenicillins Pirepacillin	Enrofloxacin 9 mg/kg q24h	28 days	118 days	negative	NA
Dog 12 (2014)	15y	F	*Escherichia coli*	No resistances	Amoxicillin/ Clavulanic acid 19 mg/kg q12h	28 days	36 days	negative	3rd culture 645 days after cessation of antimicrobial therapy: negative
Dog 13 (2015)	7y	M	*Escherichia coli*	Aminopenicillins Amphenicols Nitrofurans Polymyxins	Amoxicillin/ Clavulanic acid 18 mg/kg q12h	14 days	1 day	negative	NA
Dog 14 (2015)	9y	M castrated	*Streptococcus canis*	Tetracyclines	Enrofloxacin 11 mg/kg q24h	28 days	13 days	negative	3rd culture 127 days after cessation of antimicrobial therapy: negative

Table 3: Signalment, type of bacteria, results of antimicrobial susceptibility testing (in the 1st and 2nd urinary culture) and antibiotic treatment of the four dogs with positive follow-up culture.

Dog (year of diagnosis)	Age	Gender	Bacteria (1st culture)	Resistances against antimicrobial classes	Antibiotic therapy, type and dose	Antibiotic therapy, duration	Time to 2nd culture after cessation of antimicrobial therapy	Pathogen (2nd culture)	Resistances against antimicrobial classes
Dog 1 (2014)	13y	F spayed	*Enterobacter cloacae*	Aminopenicillins Potentiated aminopenicillins Nitrofurans	Enrofloxacin 10 mg/kg q24h	21 days	14 days	*Enterobacter cloacae*	Aminopenicillins Potentiated aminopenicillins Pirepacillin 1st- 4th generation cephalosporins Tetracyclines Fluoroquinolones Amphenicols Nitrofurans
Dog 2 (2014)	13y	F spayed	*Escherichia coli*	Sulfonamides and combination preparation (trimethoprim) Fluoroquinolones	Amoxicillin/ Clavulanic acid 21 mg/kg q12h	21 days	22 days	*Pseudomonas aeruginosa*	Aminopenicillins Potentiated aminopenicillins 1st- 4th generation cephalosporins Tetracyclines Sulfonamides and combination preparation (trimethoprim) Fluoroquinolones Amphenicols Nitrofurans
Dog 3 (2014)	11y	M castrated	*Escherichia coli*	No resistances	Amoxicillin/ Clavulanic acid 17 mg/kg q12h	14 days	28 days	*Escherichia coli*	Polymyxins

Dog 4 (2014)	6y	F spayed	*Escherichia coli*	Aminopenicillins Potentiated aminopenicillins Pirepacillin Tetracyclines Sulfonamides and combination preparation (trimethoprim) Fluoroquinolones Amphenicols	No treatment	*Escherichia coli*	Aminopenicillins Potentiated aminopenicillins Pirepacillin Tetracyclines Fluoroquinolones Amphenicols
			Klebsiella pneumoniae	Aminopenicillins Pirepacillin Nitrofurans Polymyxins		*Klebsiella pneumoniae*	Aminopenicillins Pirepacillin Nitrofurans

2.8 Figures

Figure 1: Isolated pathogens of the 29 of 161 dogs with a positive bacterial urinary culture, grouped per year of isolation. In two dogs, co-infections with two pathogens and in one dog a co-infection with three pathogens were found. Therefore, a total of 33 pathogens are listed.

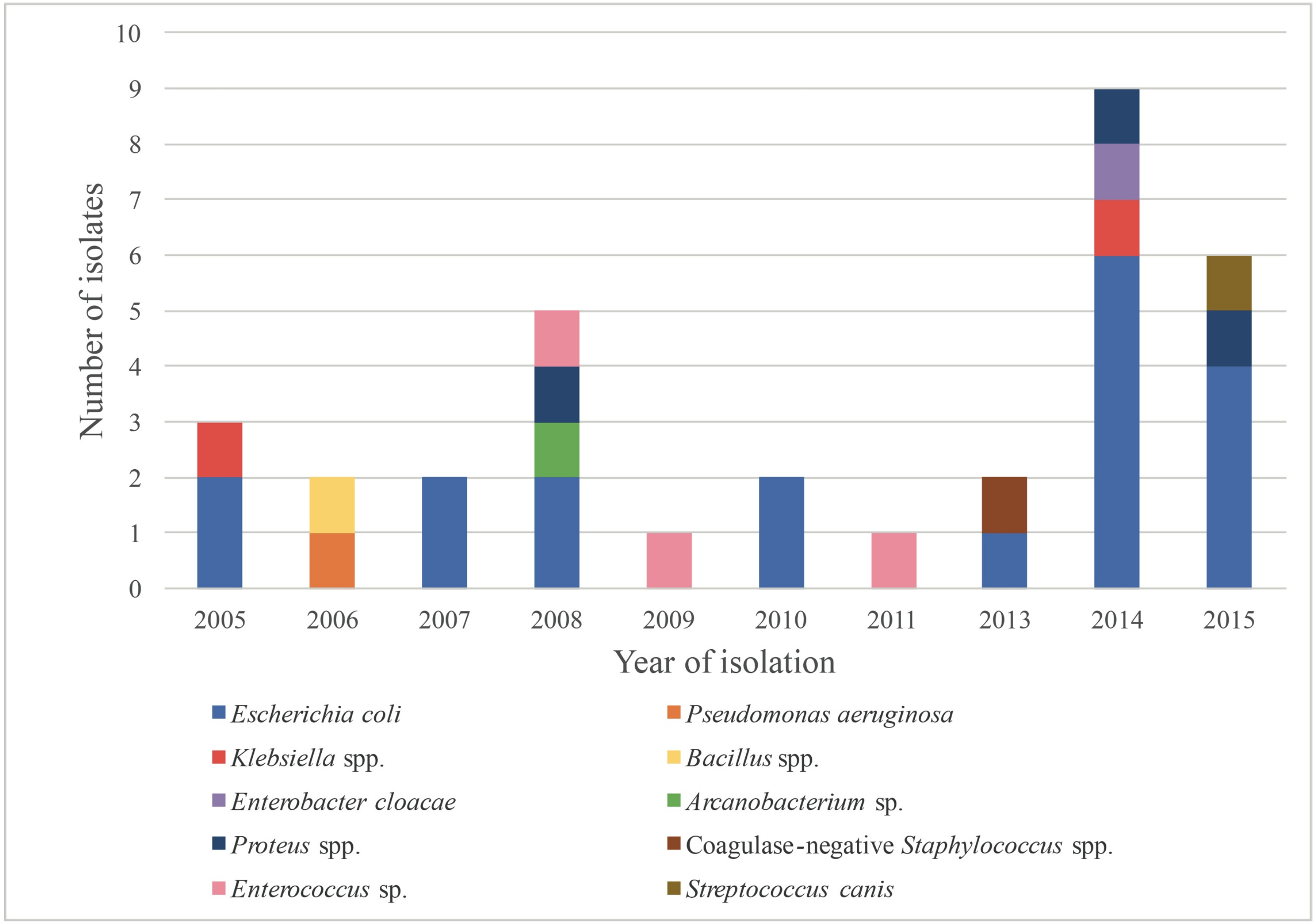
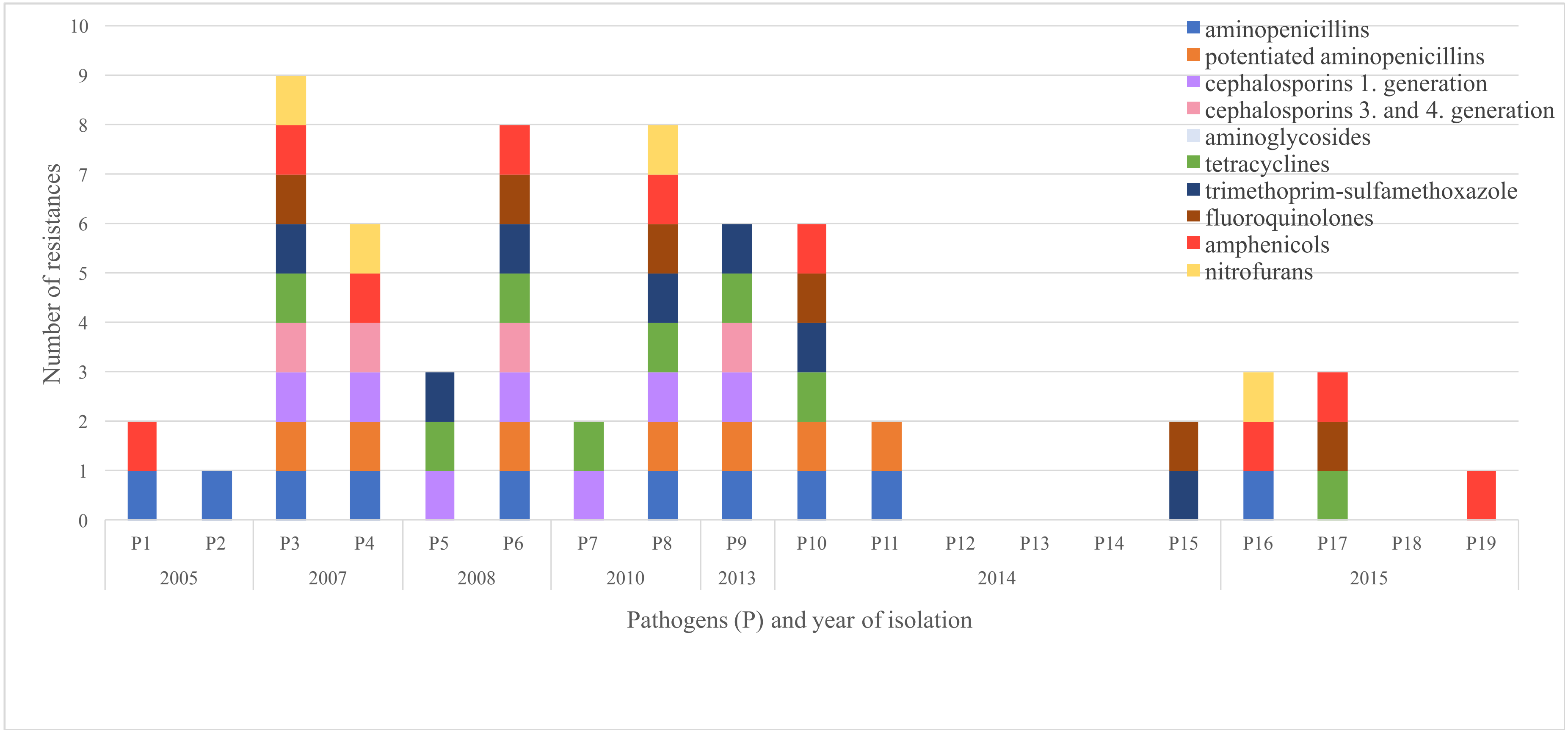


Figure 2: Number of antimicrobial resistances against 10 antimicrobial classes of the 19 *E. coli* detected in urine bacterial cultures between 2005-2015. Each pathogen is listed and pathogens are grouped per year of isolation. Abbreviation: P, pathogen.



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