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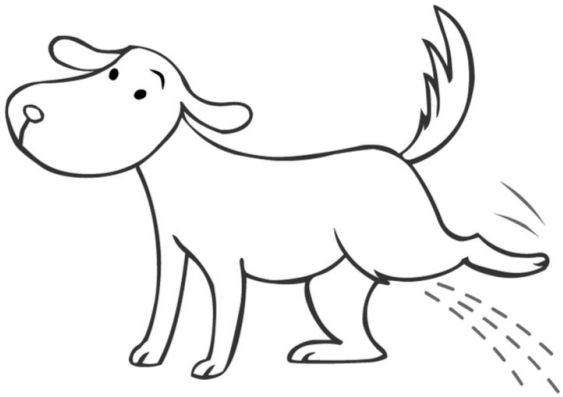
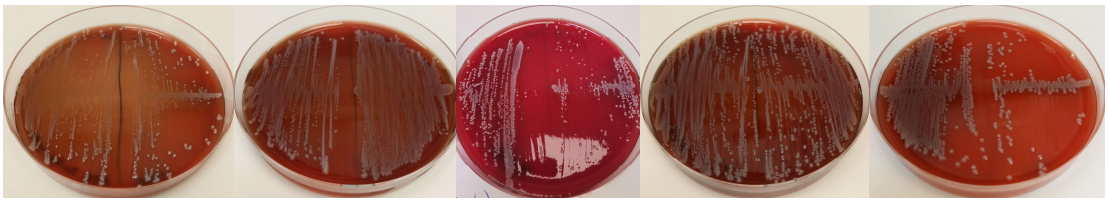
Prevalence of subclinical bacteriuria in a Danish population of dogs





Veterinary Master Thesis 2022

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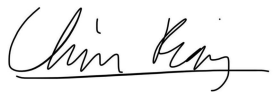
Submitted:	2nd of January 2022

Preface

This veterinary master thesis was written as a part of the final year of the Master of Science degree in Veterinary Medicine at the Faculty of Health and Medical Sciences at the University of Copenhagen.
The project was carried out from September 2021 to November 2021 at the Department of Medicine, Oncology, and Veterinary Clinical Pathology at the University of Copenhagen as a part of the initial screening of an observational study investigating long-term clinical complications on canine subclinical bacteriuria.

We would like to thank our supervisor Tina Møller Sørensen and co-supervisor Ditte Erika Leth Vasby for all your guidance, help and moral support. It is greatly appreciated. We would like to thank our co-supervisor Peter Panduro Damborg for letting us use the laboratory and materials to make the blood agar plates for our project. A thank you to the laboratory technicians for your help and guidance in preparing the blood agar plates. Also, thank you to the veterinary students and staff at the University Hospital for Companion Animals for your help along the way in recruiting patients.
We would also like to send a special thank you to Dansk Kennel Klub for your contribution to financing our project.



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Abstract

Subclinical bacteriuria is defined as a significant number of bacteria in the urine with no clinical signs of lower urinary tract disease. It is relatively new as an independent condition and lack of knowledge is leading to overuse of antibiotic treatment, as in contrast to urinary tract infections antibiotic treatment is not recommended in case of subclinical bacteriuria. Asymptomatic bacteriuria is the corresponding condition in humans, and subclinical bacteriuria in dogs is often compared to asymptomatic bacteriuria in terms of predisposing factors.

The aim of the present study was to investigate the prevalence of subclinical bacteriuria among a Danish population of dogs. The study was designed as an observational study during a 2-month period and included 120 client-owned dogs with varied health status. Bacteriologic culture of voided urine was performed as part of an initial screening. In dogs with positive cultures, subclinical bacteriuria was confirmed by antepubic cystocentesis.

The overall prevalence was 4.2% in confirmed positive dogs, and 8.3% in initially positive and confirmed positive dogs combined. Prevalence increased with age, but factors such as sex, health status, previous episodes of urinary tract infections and previous treatment with antibiotics were independent of developing subclinical bacteriuria, according to the present study.

Abbreviations

AB	Antibiotic
ABU	Asymptomatic Bacteriuria
CFU	Colony-Forming Units
EPR	Electronic Patient Records
ISCAID	International Society for Companion Animal Infectious Diseases
LUTD	Lower Urinary Tract Disease
SBU	Subclinical Bacteriuria
UTI	Urinary Tract Infection

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Introduction

Urinary tract infections (UTIs) are a common issue in dogs. It has been estimated that up to 14% of all dogs have at least one episode of UTI during their lifetime[1]. Subclinical bacteriuria (SBU) is the presence of a significant number of bacteria in the urine with no clinical signs of lower urinary tract disease (LUTD) such as pollakiuria, strangury and haematuria. SBU is relatively new as an independent condition and in contrast to other bacterial UTIs antibiotic (AB) treatment is not recommended according to the newest AB treatment guidelines from 2019[2]. According to the International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats, it requires culture of urine collected via cystocentesis to make the diagnosis of SBU[2]. *Escherichia coli* is the most common bacteria isolated from individuals with SBU. Other frequently isolated pathogens are *Staphylococcus pseudintermedius*, *Streptococcus canis*, *Enterococcus spp.* and *Klebsiella spp.*[3–5].

The research of SBU in veterinary medicine is limited and therefore it is often compared to the analogue condition asymptomatic bacteriuria (ABU) in humans. AB treatment is discouraged in most cases of ABU due to the risk of developing antibiotic-resistant bacteria[6–8]. In addition, there is no evidence of long-term complications without AB treatment, but a higher risk of symptomatic bacteriuria if treated[8,9]. Risks are assumed to be the same for dogs and thus the renewed ISCAID guidelines from 2019 recommend against AB treatment if there are no clinical signs of a UTI[2]. However, some veterinarians still prescribe AB treatment in case of a positive cultured urine sample, which may lead to over-treatment and AB resistance. Therefore, it is crucial to gather more knowledge on SBU in dogs regarding prevalence, treatment and long-term consequences.

Female dogs, older dogs and dogs with concurrent conditions or catheterization are predisposed for developing bacteriuria[10–12]. A prevalence between 2.1-8.9% of SBU has been found in healthy dogs[3,13] while higher prevalence between 9.3%*-29.9%* were found in groups of dogs with conditions such diabetes mellitus, chronic kidney disease, morbid obesity, puppies with parvovirus and dogs receiving immunosuppressants[4,5,14–19].

* Prevalence is calculated from the data of the cite sources[10,16]

The aim of this study was to investigate the prevalence of SBU among a Danish population of dogs and to identify possible risk factors for SBU. A secondary aim was to investigate if the population had any group of dogs with an increased prevalence to evaluate similarity or difference in the dogs with SBU.

The hypotheses of the study were:

1. An overall prevalence of less than 10 percent.
2. Prevalence increases with age.
3. Higher prevalence in female dogs compared to male dogs.
4. Higher prevalence in dogs with chronic disease compared to healthy dogs.
5. Higher prevalence in dogs with previous episodes of UTI.
6. Higher prevalence in dogs with previous AB treatment.

Materials and methods

Study design

The study was designed as an observational cross-sectional study approved by the Local Ethical and Administrative Committee at the Department of Veterinary Clinical Sciences.

Animals

Clinically stable dogs of any age, breed, and sex without signs of lower urinary tract infection (pollakiuria, haematuria and strangury) were recruited from September 2021 to November 2021 at the University Hospital for Companion Animals, University of Copenhagen. Clinical stability was defined as no external signs of deviations from normal hydration, blood pressure, frequency of respiration, heart rate, temperature, and oxygen saturation.

Exclusion criteria were intact males including chemically neutered males and dogs treated with systemic ABs within the last 30 days.

Recruitment

Recruitment and inclusion was performed according to the flowchart (Figure 1). In short, voided samples from eligible dogs were screened for significant bacteriuria with a quantitative urine culture.

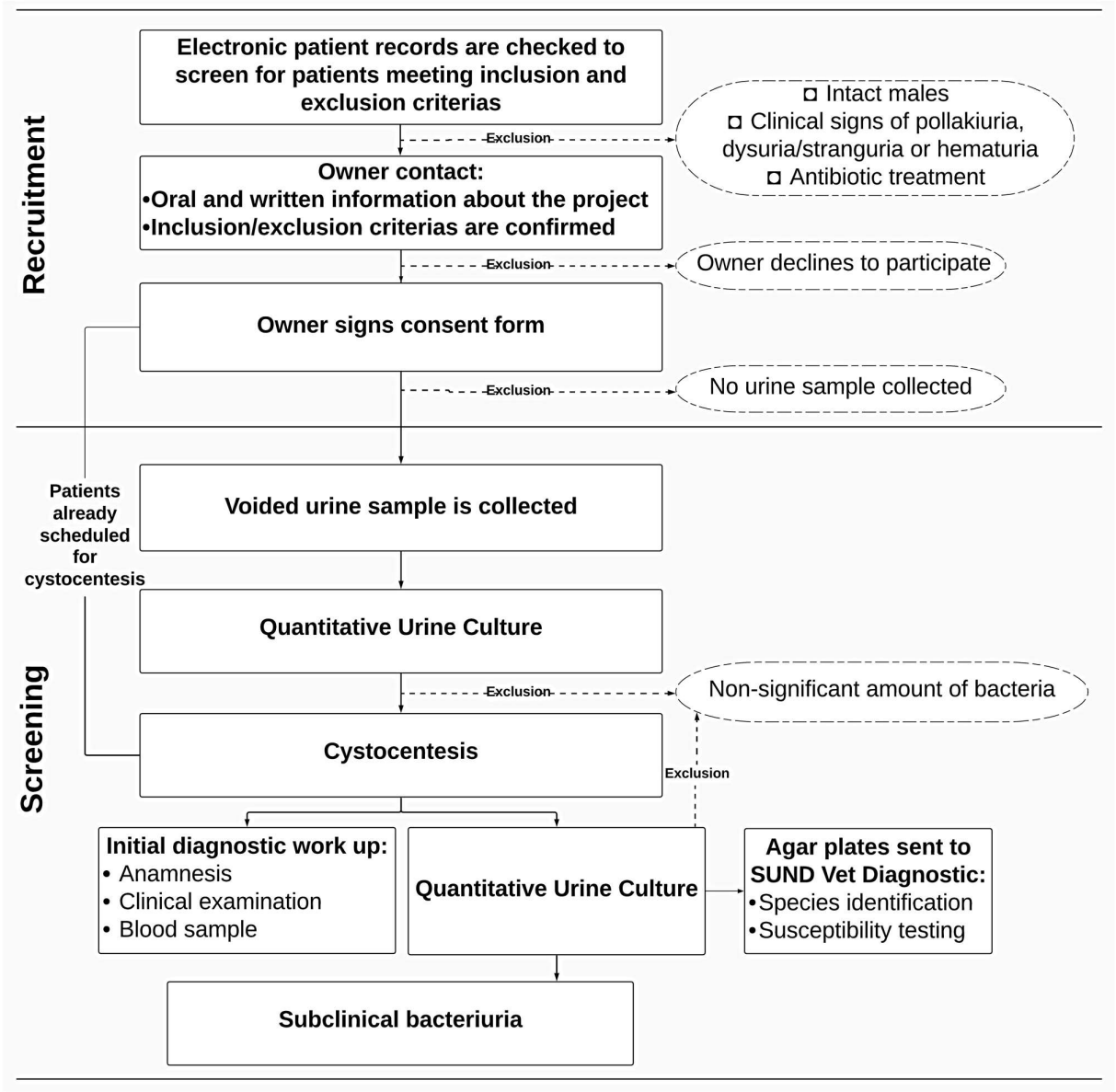

Figure 1. Flowchart illustrating the patient flow from recruitment to result of screening (Appendix A). Illustration made in lucidchart.com

Sample collection and culture

For initial screening, voided urine was collected into a sterile container (Uripet, Rocket Medical) (Appendix B) or urine was collected by ultrasound guided antepubic cystocentesis as part of scheduled diagnostic work-up (Appendix C). All urine specimens were refrigerated at 5 °C and inoculated on 5% calf blood agar plates (Appendix D) within 6 h from collection time. One µL and 10 µL of urine were streaked on each half of the plate, respectively (Figure 2). The plates were incubated at 37◦°C for 24 h (Appendix E), whereafter the colonies on each half of the plates were counted and the CFU per mL was calculated as a weighted mean[20]. Bacteriuria was considered significant when bacterial growth of ≥100.000 CFU/mL and ≥1.000 CFU/mL were present in voided and cystocentesis samples, respectively.

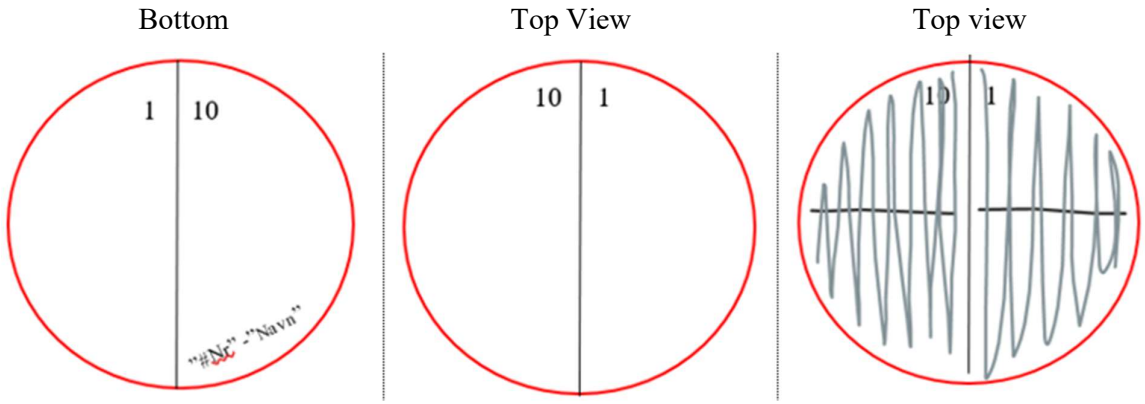

Figure 2. Illustration of blood agar plates before and after streaking.
The text on the plate refers to the number and name of the dog. The numbers 1 and 10 refer to the amount of µL that was streaked on each half. Illustration made in BioRender.com.

Further diagnostics

Dogs with significant bacteriuria at the initial screening on voided urine were asked to return for a follow-up urine sample collected by cystocentesis to rule out any false positives. If bacteriuria was confirmed, blood agar plates were sent to SUND Vet Diagnostic, UCPH, for species identification by matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry and antimicrobial susceptibility testing using the broth dilution method.

A clinical examination was performed, and clinical history was recorded. History included information of chronic conditions or conditions requiring treatment for more than 6 months, previous systemic AB treatment and previous cases of UTI (Appendix F). Information on remaining dogs was registered from the hospital's electronic patient record (EPR).

Data processing

Study size

A total study size of 300 dogs was estimated to be required to include 20 dogs with SBU for the long-term follow-up study in which the present study did the initial screening for.

Definitions prior to statistical analysis

All dogs were divided into 2 major groups according to their health status “Healthy dogs” and “Dogs with chronic disease”. “Healthy dogs” included all dogs without chronic disease including dogs with short-term disease. Two subgroups in the “healthy dogs” were made, “no disease” included clinically healthy dogs with no diagnosed conditions, and all dogs with short-term disease were grouped as “other disease”. “Dogs with chronic disease” included dogs with a disease requiring medical treatment for more than a 6-month period. A part of the dogs in the group “chronic disease” was defined as “potential risk factor disease” which included dogs with a condition that potentially raises the risk of SBU with focus on diabetes mellitus, hypercortisolism and kidney disease. Information about chronic disease was found in EPR or orally confirmed by the owner at the diagnostic follow-up.

Dogs were also divided into groups according to the results of culturing. All dogs showing non-significant bacteriuria at initial screening of urine samples were categorized as “initial negative dogs”. All dogs showing significant bacterial growth after culturing a voided urine sample were categorized as “initial positive dogs”. All dogs whose bacterial growth has been verified by culturing a sterile urine collected by cystocentesis were categorized as “SBU positive dogs”.

Statistical analysis

Comparison of significant prevalence in the groups of dogs by sex, age and presence of chronic disease were tested by 2x2 tables and Fisher’s exact test was made between the initial positive data and the population (Appendix G). Quantitative data (age) was reported as median and range and a histogram was set up for graphic display of dispersion. Descriptive methods were used for characterizing pathogens cultured for the confirmed SBU cases.

Analysis was conducted with Microsoft Excel and the program R version 4.1.1 with R Studio as statistical software. Significance level was set at $P < 0.05$.

Results

Dogs were enrolled in the study between the 28th of September to the 24th of November. Screening for SBU was offered to 186 eligible dogs, of which 121 dogs were screened. One dog was excluded retrospectively due to AB treatment within 30 days. A total of 120 dogs were included and results are summarized (Tabel 1). Twenty-two owners were not able to collect the urine sample and 43 owners declined to participate. Recruitment of patients is summarized in flowchart (Figure 3). Dogs were initially screened by culture of voided urine samples (119 dogs; 98.3%) or cystocentesis (2 dogs; 1.7%). The population included 96 purebred dogs spread on 45 different breeds and 24 dogs of mixed breed. The 3 most common dog breeds were Labrador Retriever, Golden Retriever and Cavalier King Charles Spaniel. A full list of included breeds is summarized in Appendix H.

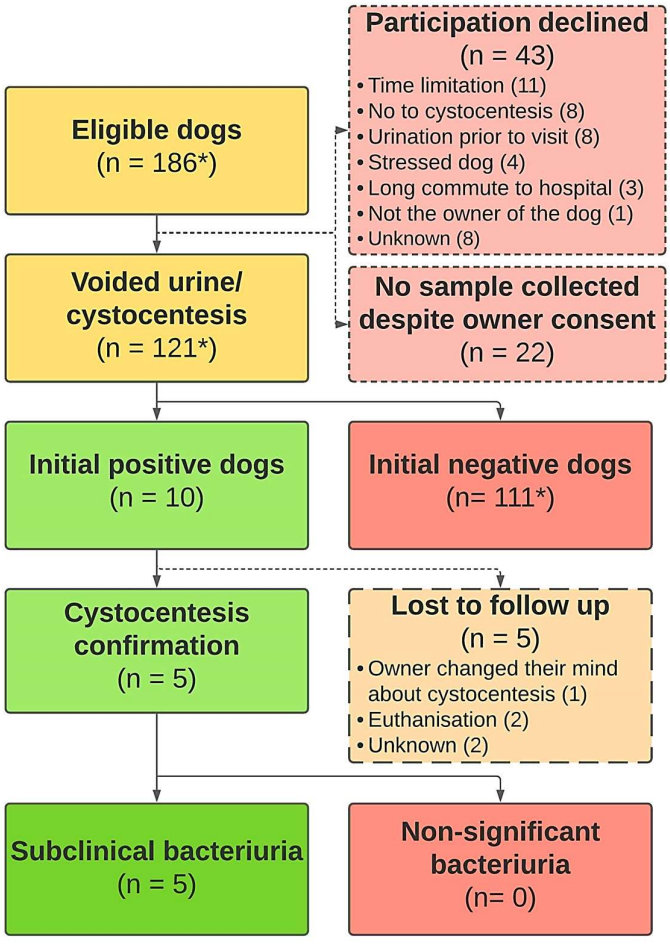

Figure 3. Flowchart illustrating the patient enrolment.

"Initial positive dogs" and "initial negative dogs" had significant bacteriuria and non-significant bacteriuria on bacteriologic culture from voided samples, respectively.

* One dog was excluded retrospectively due to antibiotic treatment within 30 days. Illustration made in lucidchart.com

Prevalence

Ten dogs (8.3%) had significant bacteriuria at initial bacterial culture. Five (4.2%) of those were confirmed by cystocentesis, while the other 5 were lost to follow-up. (Appendix I). For the dogs lost to follow-up 2 were euthanized and 3 were lost due to owner compliance.

Age

Twenty dogs were categorized as puppies (<1 year), 66 dogs as adults (1-7 years) and 34 as seniors (>7 years). Distribution of age is illustrated in Figure 4, and it shows that the data is right skewed to a age less than 2 years. Age ranged from 2 months to 14 years, and 4 years was the median. All 20 puppies were initially negative. Two adults were initially positive of which one was confirmed SBU positive. Eight seniors were initially positive of which half were also confirmed SBU positive. No significant difference in prevalence of SBU for the initially positive dogs (8.3%) over the initially negative dogs (91.7%) was found between puppies and adults (P value = 1; CI 0.00-17.80). A significant difference in prevalence of SBU were found between puppies and seniors (P value = 0.020; CI 0.00-0.88) and adults and seniors (P value = 0.0025; CI 0.010-0.57).

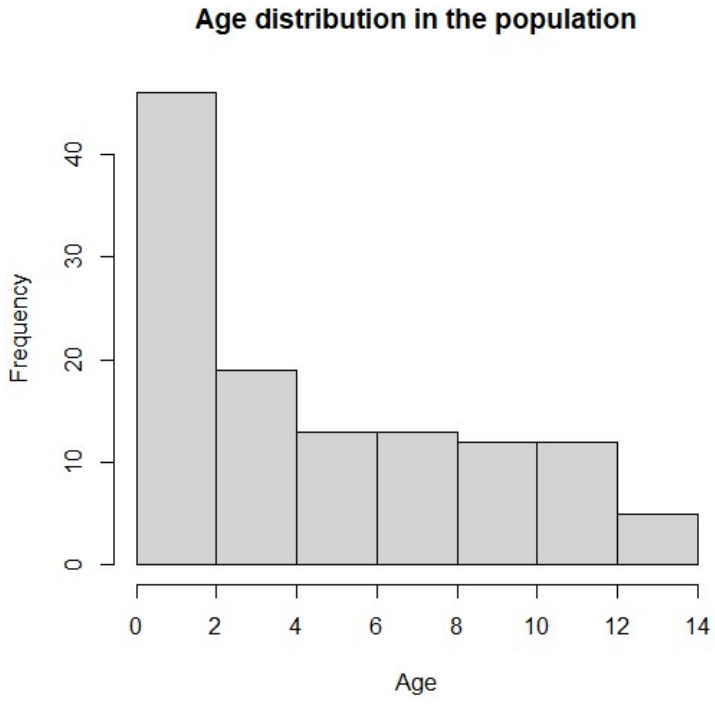

Figure 4. Histogram illustrating distribution of age in the population.

Sex

One hundred and one were female dogs (84%) and 27 (27%) of these were neutered. Eight females were initially positive of which 5 were also confirmed SBU positive. Two of the neutered females were initially positive of which 2 were confirmed SBU positive. Nineteen (16%) neutered male dogs were included. Two of the 19 males were initially positive. No significant difference in prevalence of SBU for the initially positive dogs (8.3%) compared to the initially negative dogs (91.7%) was found between females and males (P value = 0.66; CI 0.13-7.68) nor between intact and neutered females (P value = 1; CI 0.18-11.86).

Health status

Seventy-eight (65%) of the 120 included dogs were healthy without chronic disease. Of the healthy dogs, 20 (26%) were categorized as dogs with other diseases than chronic (Appendix J). Twenty (16,7%) dogs had chronic diseases, of which 2 were potential risk factors. The diagnoses of the chronic diseases are illustrated in Figure 5. Four dogs with chronic disease were initially positive of which 3 were also confirmed SBU positive. No significant difference in prevalence of SBU for the initially positive dogs (8.3%) compared to the initially negative dogs (91.7%) was found between healthy dogs, and dogs with potential risk factor disease (P value = 1; CI 0.55-14.23), or between dogs with chronic disease and potential risk factor disease (P value = 0.066; CI 0.00-23.096) nor between healthy dogs and dogs with chronic disease (P value = 0.12; CI 0.00-70.24). The calculation was based on information from 98 dogs due information on health status was not available for 22 dogs (18%).

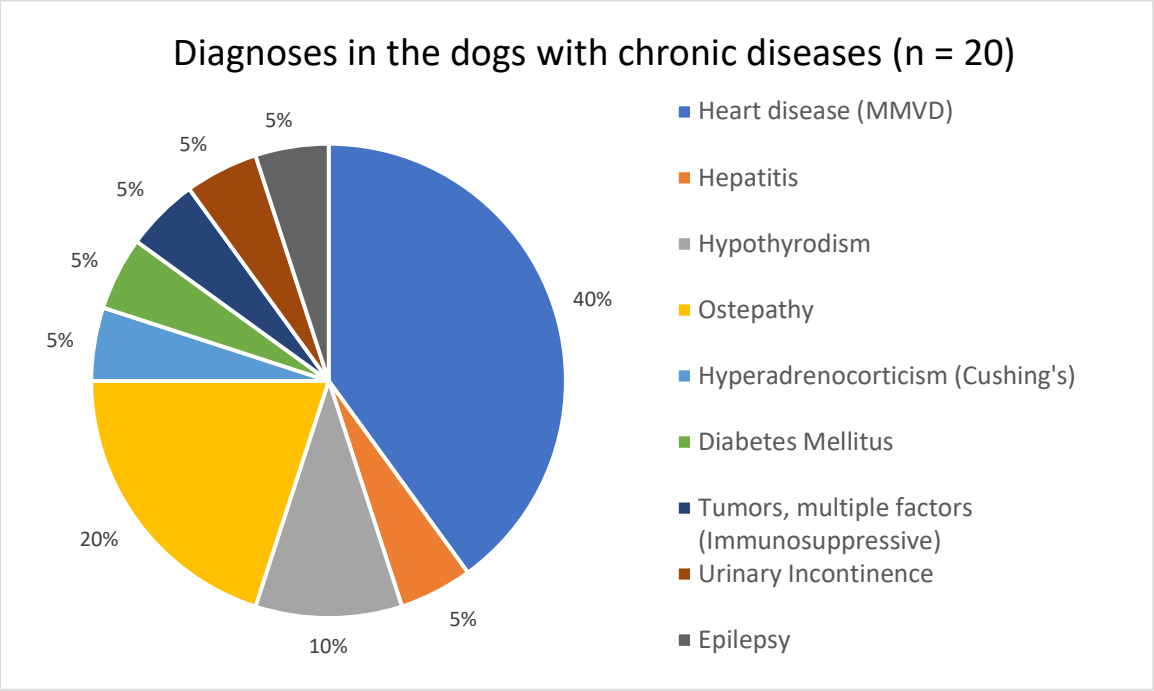

Figure 5. Distribution of the different diagnoses within the group of dogs with chronic diseases.

Previous UTI

Nine of the 120 included dogs (8%) had at least one previous episode of UTI during their lifetime. All 9 dogs were initially negative. No significant difference in prevalence of SBU for the initially positive dogs (8.3%) compared to the initially negative dogs (91.7%) was found between dogs with and dogs without previous UTI (P value = 0.59; CI 0.00-4.63). The calculation was based on information from 96 dogs due information on episodes of previous UTI was not available for 24 dogs (20%).

Antibiotic treatment

Twenty-nine of the 120 included dogs had had AB treatment during their lifetime. Of these dogs, 5 were initially positive of which 2 were also confirmed SBU positive. No significant difference in prevalence of SBU for the initially positive dogs (8.3%) compared to the initially negative dogs (91.7%) was found between dogs with and dogs without previous AB treatment (P value = 0.16; CI 0.55-12.40). The calculation was based on data from 97 dogs due information on previous treatment with systemic AB was not available for 23 dogs (19%).

Table 1 **Total data n=120 dogs** Prevalence %	All dogs (n = 120) (100%)	Healthy dogs (n = 78) (65%)	Chronic disease (n =20) (16,7%)	Initial negative (n = 110) (91,7%)	Initial positive (n = 10) (8,3 %)	SBU positive (n = 5*) (4,2%) *
Age Puppy < 1 year Adult 1-7 years Senior > 7 year	Median: 4 years Range: 2 mth - 14 years SD: 4.08 20 (17%) 66 (55%) 34 (28%)	Median: 3 years Range: 2 mth - 13 years SD: 3.52 16 (21%) 51 (65%) 11 (14%)	Median: 10 years Range: 8 mth - 14 years SD: 3.97 2 (10%) 3 (15%) 15 (75%)	Median:3,5 years Range :2 mth - 14 years SD: 3.89 20 (18%) 64 (58%) 26 (23%)	Median:10,5 years Range:7-12 years SD:1.89 0 (0%) 2 (20%) 8 (80%)	Median: 10 years Range 7-12 years SD: 1.90 0 (0%) 1 (20%) 4 (80%)
Sex Female – Sexually intact – Neutered Male (neutered)	 101 (84%) – 74 (73%) – 27 (27%) 19 (16%)	 66 (85%) – 51 (77%) – 15 (23%) 12 (15%)	 16 (80%) – 9 (56%) – 7 (44%) 4 (20%)	 93 (85%) – 68 (73%) – 25 (27%) 17 (16%)	 8 (80%) – 6 (75%) – 2 (25%) 2 (20%)	 5 (100%) – 3 (60%) – 2 (40%) 0 (0%)
Neuter status Sexually intact Neutered	 74 (62%) 46 (38%)	 51 (65%) 27 (35%)	 9 (45%) 11 (55%)	 68 (62%) 42 (38%)	 6 (60%) 4 (40%)	 3 (60%) 2 (40%)
Health status No disease Chronic disease – Potential risk factor disease Other disease NA	 58 (48%) 20 (17%) – 2 (2%) 20 (17%) 22 (18%)	 58 (74%) - - 20 (26%) 0 (0%)	 - 18 (90%) – 2 (10%) - 0 (0%)	 56 (51%) 14 (16%) – 2 (2%) 16 (15%) 22 (20%)	 3 (30%) 4 (40%) – 0 (0%) 3 (30%) 0 (0%)	 1 (20%) 1 (20%) – 0 (0%) 3 (60%) 0 (0%)
Previous UTI NA	9 (8%) 24 (20%)	8 (10%) 0 (0%)	1 (5%) 2 (10%)	9 (8%) 24 (22%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)
Previous AB NA	29 (24%) 23 (19%)	19 (24%) 0 (0%)	9 (45%) 2 (10%)	24 (22%) 23 (%)	5 (50%) 0 (0%)	2 (40%) 0 (0%)
Breed status Purebred Mixed Top breeds	 96 (80%) 24 (15%) Labrador retriever (14), Golden retriever (9), Cavalier King Charles spaniel (6)	 60 (77%) 18 (23%) Labrador retriever (11) Golden retriever (7) Cavalier King Charles Spaniel (3) French bulldog (3)	 17 (85%) 3 (15%) Border Collie (3) Cavalier King Charles Spaniel (3) Pekingese (2)	 86 (78%) 24 (22%) Labrador retriever (13) Golden retriever (9) Border Collie (4) French bulldog (4)	 10 (100%) 0 (0%) Cavalier King Charles Spaniel (4)	 5 (100%) 0 (0%) Cavalier King Charles Spaniel (2)

Table 1. Overview of data. Prevalence and distribution of prevalence according to major groups (columns) and variables (rows). NA = Not available data from this number of dogs. Percentage calculations only include available dogs.

* The 5 SBU positive dogs are included in the 10 initially positive dogs by definition.

Isolated pathogens

In the 5 confirmed SBU cases the isolated pathogens included 4 dogs with *Escherichia coli* and one dog with a mix of *Enterococcus casseliflavus* and *Staphylococcus pseudintermedius* (Appendix K).

Two dogs showed resistance to multiple antimicrobial drugs, the other three showed susceptibility to several antimicrobial drugs.

Discussion

Prevalence

The overall prevalence was 4.2% for the confirmed SBU dogs and 8.3% for the initial positive dogs. Only 5 out of 10 dogs with significant bacteriuria at the initial culture returned for a confirming cystocentesis, leaving the other half lost to follow-up. There were no false positives after cystocentesis collection. Based on the findings of another study[25], it is likely that the 5 unconfirmed dogs would also have had significant bacteriuria at follow-up. Sørensen et al 2016[25] indicate that an accurate diagnosis of SBU could be made on voided urine if the cut-off value of ≥100.000 CFU/mL was applied. This assumption is why the statistical analyses in the present study are based on the 10 initially positive dogs and why the prevalence of 8.3% is believed to be representable of dogs with SBU in the present study. The prevalence is close to the prevalence of 2.1% and 8.9% found in McGhie et al 2014[13] and Wan et al 2014[3] in a population of 140 dogs scheduled for elective surgery and 101 healthy female dogs, respectively. The major difference in method is the exclusion of dogs with predisposing factors for bacteriuria such as diabetes mellitus and immunosuppressants. This seemingly made no difference as the few that were included in the present study did not have a positive culture.

Age and health status

A higher prevalence with increasing age was found in the present study and therefore might indicate that age is a predisposing factor for SBU. Wan et al. 2014[3] did not find an increasing prevalence of SBU with age, as no significant difference in prevalence of SBU between young to middle-aged dogs (n = 6) and senior/geriatric dogs (n = 3) was found. The categorization was 1-8 year for young to middle-aged dogs and 8-14 years for senior/geriatric dogs. This contradicts the results of the present study, as a significant difference in prevalence of SBU was found between puppies and seniors (n = 0 and 8; P value = 0.020 CI 0.00-0.88) and adults and seniors (n = 2 and 8; P value = 0.0025; CI 0.010-0.57). The difference in results may be caused by the distribution of dogs in the age groups and the inclusion of puppies (<1 year) in the present study. Wan et al. 2014[3] included 51 dogs aged 1-8 years and 50 dogs aged 8-14 years compared to 66 dogs aged 1-7 years and 34 dogs aged 8-14 years in the present study. As a consequence of study design in Wan et al 2014[3], those results are more

reliable as the amount of dogs is equally distributed. In addition, no significant difference in prevalence of SBU was found between healthy dogs and dogs with chronic disease (P value = 0.12; CI 0.55-14.23). It is assumed that the frequency of chronic disease increases with age, why the correlation between SBU vs. age and SBU vs. chronic disease were expected to be more alike. It is important to keep in mind the small sample size and the distribution of age in the present study.

Studies have indicated that chronic diseases such as hyperadrenocorticism, chronic kidney disease and diabetes mellitus are potential risk factors for developing SBU[10,14,16]. The present study found no indication of this, but the sample size has likely been too small to detect these conditions. Three out of 10 initial positive dogs however had the chronic heart disease Myxomatous Mitral Valve Degeneration (MMVD). This is a common heart disease in the breed Cavalier King Charles in which the prevalence of MMVD increases with age[21]. Out of 6 enrolled Cavalier King Charles Spaniels, 4 had initially positive cultures. This study cannot with current the data differentiate which factor among age, breed or heart disease is the predisposing factor in regards to developing SBU in Cavalier King Charles Spaniels.

Sex

The urethra in women is significantly shorter than the urethra in men, which possibly explains why women are more predisposed to lower urinary tract disorders and ABU depending on age[22]. It is assumed to be the same scenario in dogs with the corresponding condition SBU. The present study found no significant difference (P value = 0.66; CI 0.13-7.68) in prevalence of SBU when comparing females and males. The lack of correlation is consistent with another study[23]. Ling et al.[23] found only a minor difference in bacteriuria comparing male and female dogs. The results do not immediately indicate that the anatomy is predisposing female dogs to developing bacteriuria.

The decision of excluding intact males was based on recommendation from ISCAID guidelines[2] saying that it is important to rule out bacterial prostatitis as the reason for bacteriuria in intact males. It was not possible to differentiate between SBU and bacterial prostatitis in the current study, due to financial constraints and the shortness of the timespan. It is likely that male dogs having acute cases of prostatitis would be excluded anyway due to signs of inflammation and clinical instability. Inclusion of all males would have contributed to a larger study size and better distribution between females and males.

Prior history of UTI and AB treatment

No significant difference in prevalence was found in dogs with or without a history of prior UTI (P value = 0.59; CI 0.00-4.63). Perhaps this is because SBU like ABU does not include pathogenic bacteria but is rather a result of a healthy urinary microbiome[8].

No significant difference in prevalence was found in dogs with or without previous systemic antibiotic treatment (P value = 0.16; CI 0.55-12.40). In humans there has been a higher occurrence of symptomatic episodes when ABU has been treated with antibiotic treatment[7,8].
None of the initial positive dogs had a history of UTI, though 5 had a history of antibiotic treatment. In the present study, there has been no distinction between different types of systemic AB treatment and therefore it is difficult to say if the treatment has influenced the urinary microbiome. The present study was not able to establish a correlation between prior UTI, SBU and AB treatment based on the study design.

In dogs as well as humans, it is not known what causes the difference between colonization and infection. There is currently no research that uncovers why bacterial colonization of the lower urinary tract does not always develop into a bacterial infection with clinical signs. Since SBU and UTI in dogs both are considered significant when growth of ≥100.000 CFU/mL and ≥1.000 CFU mL in voided and cystocentesis samples, respectively, both involve a large number of bacteria. There is no indication that SBU is a milder degree of infection than cystitis, but rather that there is a fundamental difference in the bacterial virulence. *Escherichia coli* was the most frequently isolated bacterial agent in the present study as well as in other studies of SBU[3–5]. Difference in pathogenicity of various types of Escherichia coli may explain their different ability to either cause or protect against UTI[24].

Possible biases

Information about prior antibiotic treatment, UTI and chronic disease was registered retrospectively by verbal contact with owners as the electronic records were insufficient for some dogs, particularly referred patients. This was done to limit bias, but it was not possible to gather the information about some dogs (18.3-20%) and therefore there might still be some information-bias.

In general, selection bias was limited by including all patients that came in for a consultation or a control visit (Appendix L). Dogs with impending elective surgical procedures were reachable, but owners were entitled to receive the necessary oral and written information about the study and sign

the written consent form before the urine sample collection took place. These dogs ended up not being included for logistic reasons and due to the fact that it could possibly have contributed to a bias in relation to urine concentration as anesthetized patients generally receive fluids. Dogs scheduled for elective surgery such as neutralization were typically handed over to the hospital in the early morning and picked up in the afternoon. Though it might have contributed to a slightly bigger study size, the prevalence of 2% of SBU in a study of 180 dogs presenting for elective surgery suggests that there would not necessarily have been a higher prevalence of SBU, if these were included[13].

Methods of culturing

When diagnosing ABU in humans, bacteriuria is considered significant when growth of ≥100.000 CFU/mL is found in 2 consecutive voided urine samples[22] and no requirement for a urine sample collected directly from the bladder. This distinguishes the management of ABU in humans from SBU in dogs but is possibly a result of different hygiene standards.

Nine dogs had bacterial growth between 10.000 CFU/mL and 100.000 CFU/mL on the voided urine sample (Appendix M). If the cut-off value was lower than 100.000 CFU/mL it is possible that more of the participating dogs would be diagnosed with SBU, but the risk of false positives would increase. The cut-off was set to ≥100.000 CFU/mL to minimize the chance that the bacteriuria came from bacterial contamination. Contaminating bacteria will typically be expressed by a few colonies with different expressions in morphology. The blood agar plates were all carefully inspected for contamination. In general, the colonies were very similar in morphology in cases of significant bacterial growth and the likelihood of contamination affecting the results were very small. Based on the findings in the present study it is unlikely that bacterial growth from contamination alone in 24h can lead to a significant number of bacteria or >10.000 CFU/mL.

Recruitment

A total of 43 owners declined participation (23%). The actual number might be slightly higher, as systematic registration of numbers and reasons did not start until day 4 of the recruitment phase. Two of the main reasons were busy owners and the fact that their dog had already urinated prior to the visit. In addition, 22 owners did sign the written consent, but it was not possible to obtain a urine sample the same day (12%). In most cases the owner had limited time, or the dog was anxious after the examination at the hospital and could not urinate. The odds for obtaining a urine sample might have been higher if the owner had been notified prior to the visit at the hospital. Another group of

owners declined participation because of the follow-up cystocentesis. If a similar study was conducted, it could be interesting to include only voided urine samples as this would increase owner compliance and most likely raise the number of enrolled dogs.

Conclusion

The overall prevalence of subclinical bacteriuria was 4.2% in a population of 120 dogs. This was based on the inclusion of confirmed significant bacteriuria at follow-up cystocentesis. An overall prevalence of 8.3% was found if all dogs with significant bacteriuria at initial culture of voided urine samples were included. A higher prevalence with increasing age was found and thereby the indication that age is a predisposing factor for SBU. No predisposing factors for SBU were identified in sex, health status and prior history of antibiotic treatment and UTIs.

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Appendix

Appendix A – Owner consent

KØBENHAVNS UNIVERSITET
INSTITUT FOR KLINISK VETERINÆRMEDICIN

Forekomst af bakterier i urinen hos hunde uden symptomer på blærebetændelse

Når bakterier invaderer blæren kan immunforsvaret reagere på forskellige måder, som enten resulterer i en betændelsesreaktion, det vi kender som blærebetændelse, eller i en tilstand der betegnes asymptomatisk bakteriuri hvor bakterierne lever i fredelig sameksistens med hunden uden at forårsage gener og symptomer.
Dette fænomen har været kendt og undersøgt hos mennesker i årtier, men er relativt nyt og uudforsket hos vores hunde. Derfor læner vi os op af de anbefalinger og studier, der ligger hos mennesker når det kommer til senfølger, komplikationer og behandlingsanbefalinger. Hos mennesker har man ikke kunne påvise, at der opstår senfølger relateret til asymtomatisk bakteriuri og ej heller at antibiotika har en gavnlig effekt på tilstanden eller risikoen for eventuelle komplikationer eller senfølger.

Dette forskningsprojekt ønsker at undersøge forekomsten af asymptomatisk bakteriuri hos hunde. Herudover ønsker vi at følge hunde med asymptomatisk bakteriuri i en længere periode for at undersøge om der ses senfølger af denne tilstand.

Som ejer til en hund i projektet har vi brug for at du:

- Er villig til at møde op med din hund til steril udtagning af urinprøve samt blodprøvetagning, hvis vi dyrker bakterier fra din hunds opsamlede urinprøve.
- Er villig til at møde op til mindst tre opfølgende kontrolbesøg fordelt over de næste to år. Her vil der blive foretaget urindyrkning, blodtryksmåling, og evt. blodprøve og ultralydsundersøgelse af urinvejene. Alle udgifter i forbindelse med de planlagte kontrolbesøg dækkes af projektet. Desuden tilbydes gratis dyrkning ved symptomer fra nedre urinveje i hele projektperioden.

Undertegnede erklærer hermed at have læst ovenstående, have fået besvaret eventuelle spørgsmål, og giver dermed tilladelse til, at min hund deltager i omtalte forskningsprojekt. Ved underskrift gives endvidere tilladelse til, at jeg må kontaktes i tilfælde af uddybende spørgsmål.

_____
Ejers navn

_____
Dato, ejers underskrift

_____
Hundens navn / journalnummer

Appendix B – Guide for collecting a urinesample

Opsamling af en urinprøve fra din hund

Tak for jeres deltagelse i projektet.
Dette er en vejledning om, hvordan I får opsamlet jeres hunds urinprøve.
Urin opsamlet om morgenen vil være den bedste prøve. Urinprøven må helst ikke være ældre end 6 timer af hensyn til de laboratorietest vi gerne vil udføre.
Hvis I ikke kan aflevere prøven med det samme bør den opbevares på køl (vær opmærksom på at pakke den forsvarlig og markere den tydeligt, særligt hvis du sætter den på køl i dit køleskab)

Opsamling af urinprøven

Ved brug af Uripet samles denne først og opsamlingen starter som beskrevet i punkt 2.

1. Forbered en ren, skoldet beholder, men undgå at vaske med sæbe, da det kan påvirke prøven.
2. Medbring beholderen på gåturen og luft hunden i kort line, så du er klar til at opsamle.
3. Når hunden urinerer opsamler du urinen direkte i beholderen.
4. Efter du har opsamlet urinen sættes tætsiddende låg på beholderen. Skriv gerne tid for opsamling samt navn på prøven.
5. Urinen er nu klar til at blive afleveret til os.

Collecting a urine sample from your dog

Thank you for your participation in the project.
This is a guide on how to collect your dog's urine sample.
Urine collected in the morning is considered the best sample. The collected urine should preferably not be more than 6 hours old for the sake of the laboratory test.
If you are not able to deliver the sample shortly after it has been collected it is recommended that you keep it at a cool place. (Be aware to pack the sample thoroughly and clearly mark your dogs urine sample, especially if you wish to keep the sample cool in your refrigerator overnight)

Collection of the sample

When using Uripet to collect, the first step is to assemble it and continue this guide from step 2.

1. Prepare a clean boiled container, please avoid cleaning it with soap as this can influence the sample.
2. Keep the container close when walking your dog and keep the dog at short leash so you are ready to collect the urine.
3. When the dog urinates you collect the urine directly into the container.
4. After collecting the urine you put the lid on the container and make sure it does not leak. Please write the time of collection and name upon the container.
5. Your dog's urine sample is now ready to hand in to us.

Appendix C – Protocol on cystocentesis

Cystocentesis protocol

- Equipment: A 21 to 25 gauge needle and a 6- or 12- mL syringe.
- Patient is placed in dorsal or lateral recumbency. (If needed, the patient is sedated)
- Palpate the ventral abdomen to locate the bladder rostrally to os pubis.
- Evaluate the size of the bladder as there needs to be an adequate amount of urine for cystocentesis
- The bladder is fixated with one hand at the neck of the bladder
- With a 45-degree angle the needle is inserted through the ventral abdominal wall into the bladder
- Withdraw a sufficient amount of urine and then withdraw the needle from the abdomen

References:

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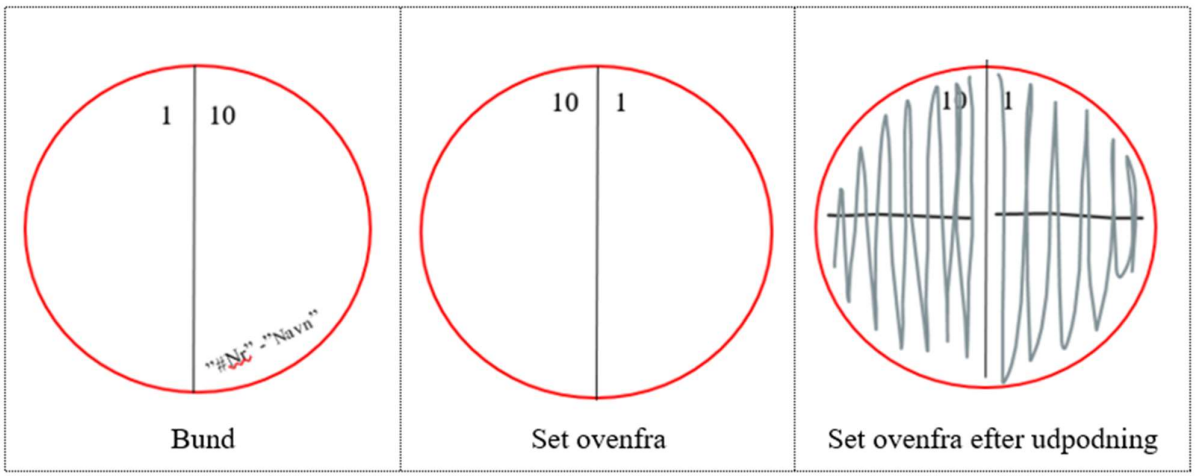
Appendix D – Protocol for production og agarplates

Protokol - fremstilling af blodagar-plader

1) KA-medie smeltes i autoklave i 30 min ved 100°C (ventilen skal åbnes). Der kan være 8 KA-flasker i ad gangen.
2) KA-flaskerne med det nu flydende KA-medie nedkøles i vandbad. Standartindstilling på vandbadet er 50°C. Det tager 30-60 min før KA-flaskerne er kølet til ca. 60°C (mellem 55-65°C).
3) Kalveblod tilsættes. Der tilsættes 10 ml i hver KA-flaske ved brug af standard-pipette (sug ekstra op og efterlad tilsvarende tilbage i pipetten for at luftbobler i agaren undgås). KA-medie og blod blandes til en ensartet masse ved at "slynge" KA-flasken blidt i luften i cirkelbevægelser. Låget åbnes og kanten flamberes over gasbrænder, før blodagaren hældes i petriskåle.
4) Blodagaren hældes i petriskåle ca. 20 ml/plade. Dette gøres ved skøn, så der bliver 10-12 blodagar-plader pr. flaske med medie. Ved bobler i agarens overflade flamberes overfladen kortvarigt, så boblerne forsvinder. Hvis der ingen bobler er i agaren, foretages ikke flambering.
6) Blodagar-pladerne stilles til side i 30-60 min., indtil de er størknet. **OBS Flyt ikke pladerne før de er helt størknet!** De tomme KA-flasker skylles og stilles klar til opvask.
7) Blodagar-pladerne præinkuberes i min. 24 timer ved 37°C. Der vedlægges navn. **HUSK Notér, hvor mange flasker og hvor mange mL blod, vi har brugt og giv besked, hvis vi bruger det sidste.**
8) Blodagar-pladerne transporteres til vores laboratorie dagen efter præinkubering, kontaminerede plader kasseres, og pladerne opbevares stablet i en tillukket pose på køl (3-5°C)

Appendix E – Protocol for culture and inoculation of urine

Dyrkning af opsamlede urinprøver på blodager



Udpladning. Dag 1:

- Før udpladning findes blodagarplader (fra køleskab) og podenåle (i kurv på hylden over varmeskab)
- Blodagarpladen opdeles i to halve og 1 µl og 10 µl noteres på hver sin halvdel. (se billede)
- På pladen noteres ligeledes ID: #nr – hundens navn. (se billede)
- Urinprøven udpodes med øjepodenål hhv. 1 µl og 10 µl på hver sin respektive halvdel.
- Podenålen føres i en stribe på tværs hvor der efterfølgende stryges over resten halvdelen. NB! tjek der podes korrekt fortynding på de to halvdele.
- Låget sættes på pladen. NB. agarpladen skal "stå på låget" så bunden med mediet er øverst.
- Blodagarpladen inkuberes aerobt ved 37° i 24 timer.

Aflæsning. Dag 2:

- Vurder om der er vækst på pladerne. Vurder om der er forskel i kolonitypen. Noter evt mistanke om kontamination.
- Tæl antal kolonier (op til 100) for hver halvdel. Noter antal i skema (Flere end 100 noteres ">100").
- Udregn CFU/mL: $\frac{Antal\ kolonier}{11} x\ 1000$
- Tag billede af prøver med vækst. Disse uploades til foto-mappen (onedrive).

Mistænk kontamination og noter dette hvis:

- Vækst ses på et område der ikke er podet
- Der ses flere kolonityper (1+)
- Markant forskel i vækst på de to halvdele. Kontamination ved mindre end x5 kolonier på 10 µl halvdelen end 1 µl halvdelen.

Grænseværdier (*DDD AB-anbfalinger*)	
Udtagningsmetode	**Hund**
Spontan urinprøve	> 100.000 CFU/mL
Cystocentese	> 1.000 CFU/mL

Appendix F – Anamnesis for clinical examination

Anamnese-skema og klinisk undersøgelse ifbm. cystocentese besøg

Anamnese (incl. specifik for projekt)**:**	**Screening for subklinisk bakteriuri efter positiv dyrkning på opsamlet prøve**
Almentilstand samt aktivitetsnivau:	
Smerter/smerteytringer:	
Fodertype (ex fuldfoder, hjemmefoder, sygdomsdiæt) og fodring:	
Æde-/drikkelyst:	
Urin/urinering: +ændringer? ex besværet el. ukontrolleret urinering, hyppighed +urin vurdering? ex farve, blod, lugt	
Fæces/defækation:	
Reproduktionshistorik han/hun (seneste løbetid etc.):	
Rejsehistorik	
Tidligere diagnosticeret urinvejsinfektion (hvornår? flere gange?)	
Sygdomme/medicinforbrug: +medicin som glukokortikoider/prednisolon, antibiotika (30dage) +stofskiftelidelser, hyperthyroidisme, hyperadrenocorticisme, diabetes mellitus (sukkersyge) el. nyresygdom	

Appendix G – Calculations for Fisher test

Statistical significance of association

for the 10 initial positive by voided urine sample

p-values Fischer test		
comparison	**p-value**	**interpretation**
male vs female	0,6581	non-significant
female intact vs female neutered	1	non-significant
puppy vs adult	1	non-significant
puppy vs senior	0,0201	significant
adult vs senior	0,002457	significant
healthy vs chronic disease	0,1166	non-significant
healthy vs risk factor disease	1	non-significant
chronic disease vs risk factor disease	1	non-significant
UTI vs no UTI	0,5908	non-significant
AB vs no AB	0,1594	non-significant

Code on matrix (2x2) and Fischer test in R Studio

Male vs female

```
> sex <- matrix(c(8,2,93,17), nrow = 2)
> row.names(sex) <- c("female all", "male neutered")
> colnames(sex) <- c("SBU positive", "SBU negative")
> sex
              SBU positive SBU negative
female all               8           93
male neutered            2           17
> fisher.test(sex)

        Fisher's Exact Test for Count Data

data:  sex
p-value = 0.6581
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.1296579 7.6752978
sample estimates:
odds ratio
 0.7332686

>
```

Female intact vs female neutered

```
> female <- matrix(c(6,2,68,25), nrow = 2)
> row.names(female) <- c("female intact", "female neutered")
> colnames(female) <- c("SBU positive", "SBU negative")
> female
                SBU positive SBU negative
female intact              6           68
female neutered            2           25
> fisher.test(female)

        Fisher's Exact Test for Count Data

data:  female
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.1811628 11.8629765
sample estimates:
odds ratio
   1.10189

>
```

Puppy vs adult

```
> age <- matrix(c(0,2,20,64), nrow = 2)
> row.names(age) <- c("puppy", "adult")
> colnames(age) <- c("SBU positive", "SBU negative")
> age
       SBU positive SBU negative
puppy             0           20
adult             2           64
> fisher.test(age)

        Fisher's Exact Test for Count Data

data:  age
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.00000 17.79533
sample estimates:
odds ratio
         0

>
```

Puppy vs senior

```
> age <- matrix(c(0,8,20,26), nrow = 2)
> row.names(age) <- c("puppy", "senior")
> colnames(age) <- c("SBU positive", "SBU negative")
> age
       SBU positive SBU negative
puppy             0           20
senior            8           26
> fisher.test(age)

        Fisher's Exact Test for Count Data

data:  age
p-value = 0.0201
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.0000000 0.8819748
sample estimates:
odds ratio
         0

>
```

Adult vs senior

```
> age <- matrix(c(2,8,64,26), nrow = 2)
> row.names(age) <- c("adult", "senior")
> colnames(age) <- c("SBUpositve", "SBUnegative")
> age
       SBUpositve SBUnegative
adult           2          64
senior          8          26
> fisher.test(age)

        Fisher's Exact Test for Count Data

data:  age
p-value = 0.002457
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.01012595 0.56940145
sample estimates:
odds ratio
 0.1041689

>
```

Healthy vs chronic disease

```
> disease <- matrix(c(4,6,16,72), nrow = 2)
> row.names(disease) <- c("chronic disease", "healthy")
> colnames(disease) <- c("SBU positive", "SBU negative")
> disease
                SBU positive SBU negative
chronic disease            4           16
healthy                    6           72
> fisher.test(disease)

        Fisher's Exact Test for Count Data

data:  disease
p-value = 0.1166
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.5487403 14.2375269
sample estimates:
odds ratio
  2.957754

>
```

Healthy vs risk factor disease

```
> disease <- matrix(c(0,6,2,72), nrow = 2)
> row.names(disease) <- c("risk", "healthy")
> colnames(disease) <- c("SBU positive", "SBU negative")
> disease
        SBU positive SBU negative
risk               0            2
healthy            6           72
> fisher.test(disease)

        Fisher's Exact Test for Count Data

data:  disease
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.00000 70.23794
sample estimates:
odds ratio
         0

>
```

Chronic disease vs risk factor disease

```
> disease <- matrix(c(0,4,2,14), nrow = 2)
> row.names(disease) <- c("risk", "chronic disease")
> colnames(disease) <- c("SBU positive", "SBU negative")
> disease
               SBU positive SBU negative
risk                      0            2
chronic disease           4           14
> fisher.test(disease)

        Fisher's Exact Test for Count Data

data:  disease
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.00000 23.09556
sample estimates:
odds ratio
         0

> |
```

UTI vs no UTI

```
> UTI <- matrix(c(0,10,9,77), nrow = 2)
> row.names(UTI) <- c("UTI", "no UTI")
> colnames(UTI) <- c("SBU positive", "SBU negative")
> UTI
       SBU positive SBU negative
UTI               0            9
no UTI           10           77
> fisher.test(UTI)

        Fisher's Exact Test for Count Data

data:  UTI
p-value = 0.5908
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.000000 4.626613
sample estimates:
odds ratio
         0

> |
```

AB vs no AB

```
> AB <- matrix(c(5,5,24,63), nrow = 2)
> row.names(AB) <- c("AB", "no AB")
> colnames(AB) <- c("SBU positive", "SBU negative")
> AB
      SBU positive SBU negative
AB               5           24
no AB            5           63
> fisher.test(AB)

        Fisher's Exact Test for Count Data

data:  AB
p-value = 0.1594
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.5450152 12.3969950
sample estimates:
odds ratio
  2.595564

> |
```

Appendix H – List of dog breeds

Purebred	Frequency
Alaskan Klee Kai	1
Australsk Shepherd	2
Beagle	2
Bichon Havanais	3
Border Collie	4
Brichon Frise	1
Broholmer	1
Cavalier King Charles Spaniel	6
Chihuahua, langhåret	1
Cocker Spaniel	2
Cotton de Tulear	1
Dansk Svensk Gårdhund	4
Dogue de Bordeaux	1
Dværgpuddel	1
Dværgschnauzer	1
Engelsk Cocker Spaniel	1
Eurasier	1
Fransk Bulldog	4
Golden Retriever	9
Gravhund	2
Irish Softcoat Wheaten Terrier	1
Jack Russel Terrier	2
Japansk Spids	2
Kinesisk Hårløs	1
Kleiner Munsterlænder	2
Labrador Retriever	14
Lagotto Romagnolo	1
Lhasa Apso	1
Malteser	1
Mops	2
Nova Scotia Duck Tolling Retriever	1
Old English Bulldog	1
Pekingeser	2
Perro de Agua Espanol	1
Pomeranien	2
Puddel	1
Samojedehund	3
Sankt Bernhardshund, Korthåret	1
Sealyham Terrier	1
Shih Tzu	2
Staffordshire Bull Terier	2
Weimaraner	1
Welsh Corgi Pembroke	1
West Highland Terirrer	1
Yorkshire Terrier	1
SUM	96

Mixed breed	Frequency
Blanding	1
Blanding, Lille	4
Blanding, Mellem	10
Blanding, Stor	3
Cavapoo	1
Cockerpoo	2
Labradoodle	3
SUM	24

Appendix I – Calculation of prevalence

Prevalence for the population:

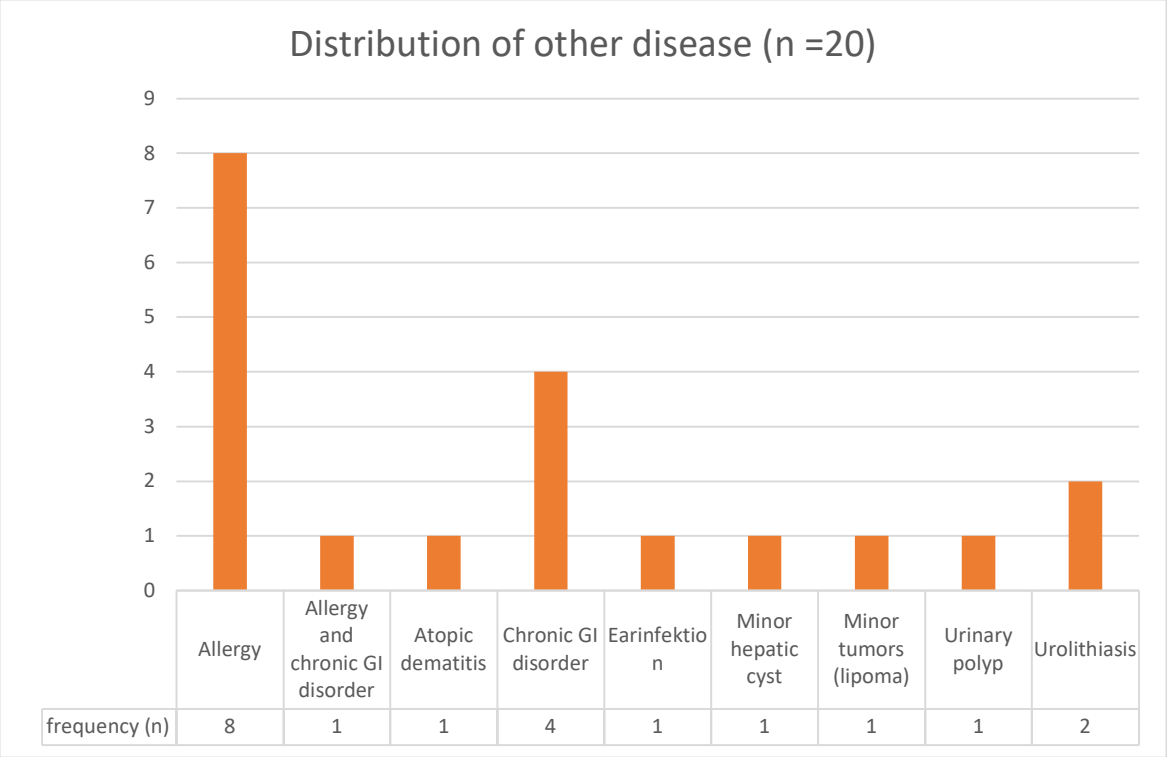
Prevalens = #SBU positives / #total population

Antal SBU positive	10		
Total population	120		
Prevalens	0,0833	**8,33%**	**by voided**
Nedre 95% konfidensintervalgrænse (tilnærmet)	0,0339	3,39%	
Øvre 95% konfidensintervalgrænse (tilnærmet)	0,1328	13,28%	

Antal SBU positive	5		
Total population	120		
Prevalens	0,0417	**4,17%**	**by cysto**
Nedre 95% konfidensintervalgrænse (tilnærmet)	0,0059	0,59%	
Øvre 95% konfidensintervalgrænse (tilnærmet)	0,0774	7,74%	

Appendix J - Distribution of other diseases in the population

Chart showing the distribution and diagnoses in the category "other disease"



	Allergy	Allergy and chronic GI disorder	Atopic dematitis	Chronic GI disorder	Earinfektion	Minor hepatic cyst	Minor tumors (lipoma)	Urinary polyp	Urolithiasis
frequency (n)	8	1	1	4	1	1	1	1	2

*Chronic GI disorder includes all GI disorders such as the diagnosis Inflammatory Bowel Disease, IBD
*GI = gastrointestinal

Appendix K – List of isolated pathogens

Isolated pathogens:

Table of agens SBU confirmed dogs

Dog	Isolated pathogen	Sensitivity for treatment
38: Nelly	E. coli (single culture)	S to all (NI til Cefovecin)
46: Betha	E. coli (single culture)	S to all (NI til Cefovecin)
5: Flora	E. coli (single culture)	S to all except I to Cefazolin (NI til Cefovecin)
51: Bisquit	E. coli (single culture, possible ESBL)	R to ampicillin, Cefalexin, Cefazolin, Cefpodoxime, Ceftazidime, Gentamicin og Trimethoprim/Sulfamethoxazole (NI to Cefovecin)
95: Joline	Mix of Enterococcus casseliflavus og S. pseudointermedius	E. casseliflavus: R to Amikacin, Cefazolin, Cefovecin, Cefpodoxime, Cephalothin, Gentamicin, Rifampin og Vancomycin. I to Erythromycin. S. pseudointermedius: S to alle

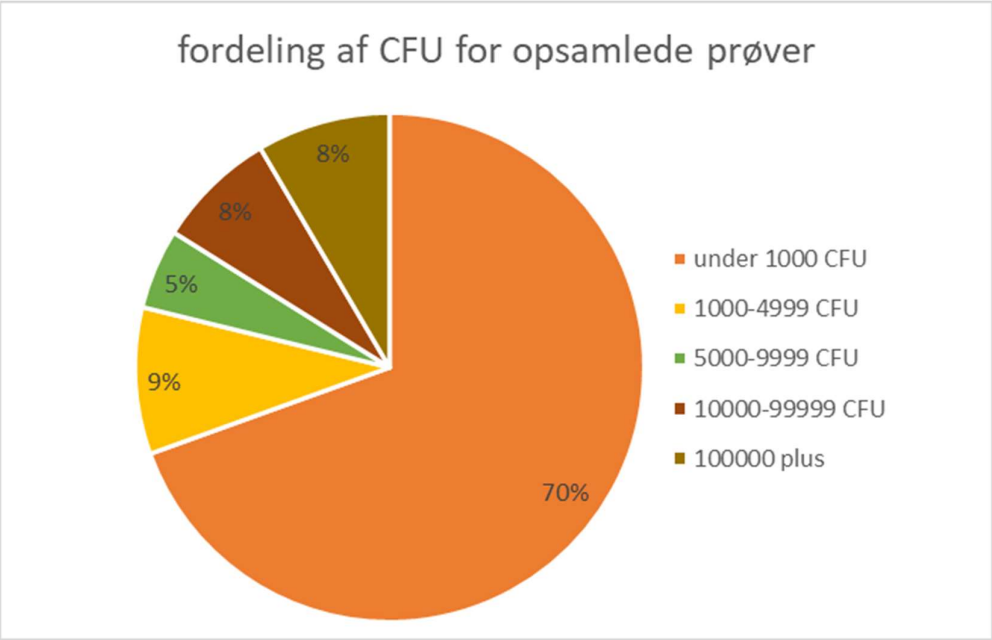
*NI - No Interpretation, R - Resistant (treatment not possible), S - Susceptible (treatment possible), I - Intermediate (treatment possible if dosage is increased or drug accumulates at the infection site)

Appendix L – Recruitment services

Hospital tracks	patients recruitet (n)	%
almen KIR	2	1,7
dermatologi	12	10,0
extern	3	2,5
fysioterapi	2	1,7
intern medicin	10	8,3
kardiologi	2	1,7
kir konsultation	4	3,3
modtagelsen	63	52,5
onkologi	2	1,7
orthopædi	2	1,7
røntgen	2	1,7
slankeklinik	1	0,8
smerteklinik	7	5,8
soft tissue	6	5,0
ultralyd	2	1,7

top 3 tracks	modtagelsen (63), dermatologi (12), intern medicin (10)		
top 3 tracks	85	70,8	%
other tracks	35	29,2	%
Distribution of top 3 tracks			
modtagelsen	74	%	
dermatologi	14	%	
intern medicin	12	%	

Appendix M – Distribution of CFU/mL



under 1000 CFU	82
1000-4999 CFU	11
5000-9999 CFU	6
10000-99999 CFU	9
100000 plus	10