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der Vetsuisse-Fakultät Universität Zürich

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Carriage of methicillin-resistant *S. aureus* and *S. pseudintermedius*, ESBL-producing or carbapenemase-producing *Enterobacteriaceae* in dogs with hypercortisolism

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

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genehmigt auf Antrag von

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2018

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Vetsuisse-Fakulty University of Zurich (2018)

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Carriage of methicillin-resistant *S. aureus* and *pseudintermedius*, ESBL-producing or carbapenemase-producing *Enterobacteriaceae* in dogs with hypercortisolism

The importance of antimicrobial resistant bacteria has increased significantly in small animal medicine. We investigated methicillin-resistant *S. aureus* and *S. pseudintermedius*, ESBL-producing or carbapenemase-producing *Enterobacteriaceae* carriage in dogs with hypercortisolism (HC), which are considered immunocompromised and in healthy dogs. Based on our results, dogs with HC are at increased risk for carriage of ESBL-PE, and carriage rate seemed higher in HC dogs receiving a raw food diet.

Keywords: antibiotic resistance; canine; swab sampling; cushing syndrome

Trägerschaft von methicillin-resistenten *S. aureus* und *pseudintermedius*, ESBL- oder Carbapenemase-produzierende *Enterobacteriaceae* bei Hunden mit Hyperkortisolismus

Die Bedeutung antimikrobiell resistenter Bakterien hat in der Kleintiermedizin signifikant zugenommen. Wir haben die Trägerschaft von methicillin-resistenten *S. aureus* und *S. pseudintermedius*, ESBL- oder Carbapenemase-produzierende *Enterobacteriaceae* bei Hunden mit Hyperkortisolismus (HK), die als immunsuppremiert gelten, und bei gesunden Hunden untersucht. Basierend auf unseren Ergebnissen besteht für Hunde mit HK ein erhöhtes Risiko für eine Trägerschaft mit ESBL-PE, und diese schien bei Hunden mit HK, die eine Rohfleischfütterung erhielten, höher zu sein.

Stichworte: Antibiotikaresistenz; Hund; Tupferproben; Cushing Syndrom

Carriage of methicillin-resistant *S. aureus* and *S. pseudintermedius*, ESBL-producing or carbapenemase-producing *Enterobacteriaceae* in dogs with hypercortisolism

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Keywords: antimicrobial resistance; canine; swab sampling; cushing syndrome

List of abbreviations:

AMR: antimicrobial resistant

HC: hypercortisolism

MRSA: methicillin-resistant *Staphylococcus aureus*

MRSP: methicillin-resistant *Staphylococcus pseudintermedius*

ESBL-PE: extended-spectrum β-lactamase-producing *Enterobacteriaceae*

CPE: carbapenemase-producing *Enterobacteriaceae*

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The work was done at the Clinic of Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

Grants: This research was supported by the Research Fund of the University of Zurich 2016.

Conflict of Interest Declaration: The authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

Institutional animal care and use committee (IACUC) or other approval: This study was approved by the veterinary office of the canton of Zurich and is in accordance with the guidelines and directives established by the Animal Welfare Act of Switzerland (TVB 168/2016). Informed consent was obtained from every owner.

Acknowledgments: The authors gratefully acknowledge the veterinarians of the Clinic for Small Animal Internal Medicine for their contribution of cases. We thank all dog owners for their assistance and willingness to take part in this study and Marina Morach for her help with the laboratory work.

Marked for review:

Deleted on page 4, line 76: Additional reference: Sieber-Ruckstuhl et al., Abstract 27th ECVIM-CA congress 2017.

Deleted on page 5, line 94: Manufacturer of β LACTA™ test kit BioRad, Cressier, Switzerland.

Abstract

Background: The importance of antimicrobial resistant (AMR) bacteria has increased significantly in small animal medicine. AMR bacteria can represent a zoonotic risk for humans. Dogs with hypercortisolism (HC) are often immunocompromised, which could increase the risk of bacterial carriage or infection. So far, no studies have investigated carriage of methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-PE) and carbapenemase-producing *Enterobacteriaceae* (CPE) in dogs with HC. **Objectives:** To investigate MRSA, MRSP, ESBL-PE, CPE carriage in dogs with HC and in healthy dogs and to link raw food diet and hospitalization as potential risk factors. **Animals:** 38 client-owned dogs with naturally occurring HC and 12 aged-matched client-owned healthy dogs. **Methods:** Prospective clinical study. Four swab samples from typical sites of carriage (nasal, pharyngeal, 2 rectal) were analyzed at the time of inclusion into the study and 1-3 times thereafter. Swabs were cultured for the occurrence of MRSA, MRSP, ESBL-PE and/or CPE. **Results:** Four dogs with HC (10.5%), but none of the healthy dogs tested positive for carriage (rectal swabs) of ESBL-PE. There was a significant association of feeding a raw food diet and carriage of ESBL-PE in dogs with HC, but not in healthy dogs. **Conclusion and clinical importance:** This study gives evidence that dogs with HC are at increased risk for carriage of ESBL-PE. Feeding a raw food diet seemed to increase the ESBL-PE carriage rate in dogs with HC. More studies evaluating AMR bacteria carriage and infections in dogs with HC and healthy dogs are needed.

Introduction

Companion animals are increasingly important in modern society and the human-animal-bond is more and more intense. This close contact between owner and animal leads to early recognition of health problems and early presentation of animals to veterinarians. Bacterial infections are common in companion animals and the use of antimicrobials is part of daily business in a small animal practice. Unfortunately, increased antibiotic use is one known factor in the development of antimicrobial resistant (AMR) bacteria.

Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-resistant S*taphylococcus pseudintermedius* (MRSP), extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-PE) and, more recently, carbapenemase-producing *Enterobacteriaceae* (CPE) have emerged in both healthy and sick animals.[1-8]

Transmission of these bacteria from infected or colonized companion animals to humans is a real threat and therefore a key public health concern.[2,5-14]

Hypercortisolemia (naturally occurring or related to glucocorticoid therapy) induces a state of immunocompromise and increases susceptibility to various pathogens.[15,16] Dogs receiving glucocorticoids were shown to have an increased risk for MRSP carriage.[17] However, nothing is known about the risk of MRSP or other AMR bacteria carriage in dogs with naturally occurring hypercortisolism (HC). Feeding a raw food diet and prior hospitalization have been shown to increase the risk for MRSP and ESBL-PE carriage in dogs.[17-19] Whether this holds true for dogs with naturally occurring HC is not known.

Thus, the aim of the present study was to investigate the carriage rate of MRSA, MRSP, ESBL-PE and CPE in dogs with naturally occurring HC as well as that of healthy dogs. Furthermore, feeding a raw food diet and hospitalization were investigated as possible risk factors for MRSA, MRSP, ESBL-PE and CPE carriage.

Materials and Methods

Animals

Client-owned dogs with naturally occurring HC were prospectively enrolled. Dogs were either included at the time of diagnosis of HC or during re-evaluation of trilostane therapy (Vetoryl®, Dechra, Europe). The diagnosis of HC was based on a combination of history, physical examination findings, haematology, serum biochemistry profile, urinalysis and positive endocrine testing (low-dose dexamethasone suppression test and/or ACTH

stimulation test).[20] Dogs were excluded from the study if they had a history of antimicrobial therapy within two weeks prior to the time of enrolment.
Healthy, aged-matched, client-owned dogs were enrolled as controls. The dogs were considered healthy, on the basis of detailed information provided by their owners and the results of a physical examination, haematology, and serum biochemistry profile.

Experimental design

The study was performed between October 2016 and February 2018 at our clinic. In order to evaluate carriage of MRSA, MRSP, ESBL-PE and/or CPE, sterile cotton swabs moistened with sterile saline were used to collect nasal (number of swabs/dog: 1), pharyngeal (1) and rectal samples (2). Swab sampling was performed at the time of inclusion (T0) in all dogs and if possible 1-3 times thereafter (T1-T3) in dogs with HC. Nasal swabs were collected by inserting the swabs 0.5 cm into the nostrils; pharyngeal swabs by swiping them across the pharyngeal region; and rectal swabs by inserting them 0.5-1 cm into the rectum. All swab samples were collected in the morning immediately after clinical examination of the dogs. During the initial examination, owners were questioned about prior hospitalisation (>24 hours) and about the diet of their dogs over the previous 4 weeks.
Dogs with newly diagnosed HC were started on trilostane therapy (starting dose 0.5-1 mg/kg q12h) after inclusion in the study. To monitor trilostane therapy, cortisol concentrations were measured twice, 1 hour apart, just before the morning trilostane dose (prepill 1 and prepill 2 cortisol).[21] Every dog undergoing trilostane therapy was assessed by standardized owner questionnaire and by clinical examination. The trilostane dose was adjusted according to the prepill cortisol concentrations and the clinical signs.

Bacteriological analysis

All swabs were placed in sterile plain tubes until processing. The nasal, and pharyngeal swabs and one rectal swab were pre-enriched in Mueller-Hinton Broth (Oxoid, Hampshire, UK) with 6.5 % NaCl. Thereafter, 1ml was transferred to 9 ml Tryptic soy broth with cefoxitin (3.5mg/L) and aztreonam (75mg/L). After incubation at 37 °C for 16-20h, a total of 10 µl was transferred onto Brilliance MRSA 2 Agar (Oxoid, Hampshire, UK). Presumptive positive colonies were identified using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany). The second rectal swab was pre-enriched in *Enterobacteriaceae* Enrichment broth (BD, Franklin Lakes, USA).

After incubation at 37 °C for 16-20 h, a total of 10 µl was transferred on Oxoid Brilliance™ ESBL Agar (Oxoid, Hampshire, UK), and onto chromID Carba Smart Agar (Bio Merieux, Marcy-l'Etoile, France). Presumptive positive colonies on Oxoid Brilliance™ ESBL Agar were verified for ESBL production with the colorimetric β LACTA™ test kit (BioRad), according to the manufacturer's instructions. Presumptive positive colonies on chromID Carba Smart Agar were confirmed as Carbapenemase-producers by the NP Carba test (Bio Merieux, Marcy-l'Etoile, France), according to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using commercially available software (GraphPad Prism5, Graph Pad Software, San Diego, CA, USA). Differences in MRSA, MRSP, ESBL-PE, CPE carriage were tested by means of the Fisher exact test. The level of significance was set at $p < 0.05$.

Results

Animals

Thirty-eight dogs with HC were included in the study. Age ranged from 2.8-15.1 years (median, 10.6) and body weight from 2.9-38.8 kg (median, 8.9). Twenty-one dogs were male (11 castrated) and 17 were female (14 spayed). There were 31 pure-breed and 7 mixed-breed dogs. Twelve dogs were newly diagnosed with HC and 26 dogs were on trilostane therapy when included into the study.

Twelve healthy dogs were included as a control group. Age ranged from 5.7-14 years (median, 8.1) and body weight from 7.5-44.5 kg (median, 22.1). Five dogs were male (4 castrated) and 7 were female (5 spayed). There were 9 pure-breed and 3 mixed-breed dogs.

Initial sampling

Results of carriage of MRSA, MRSP, ESBL-PE, CPE of healthy dogs and dogs with HC at the time of enrolment are summarized in table 1.

ESBL-producing *Enterobacteriaceae* were found in rectal swabs from four dogs with HC (10.5%; 1 newly diagnosed dog, 3 dogs on trilostane therapy (length of trilostane therapy: 1, 3 and 19 months)).

None of the healthy dogs showed carriage of MRSA, MRSP, ESBL-PE, CPE.

Sampling during follow-up in dogs with HC

Results for MRSA, MRSP, ESBL-PE, CPE carriage in dogs with HC during follow up are summarized in table 2. Follow up sampling was performed between 1.6-8.8 months (median, 4.5 months) (T1), 4.3-10.8 months (median, 7.5 months) (T2) and 9.6-15.6 months (median, 13 months) (T3) after inclusion in the study, depending on the individual recheck time of each dog.

None of the initially negative dogs tested positive for MRSA, MRSP, ESBL-PE, CPE carriage during the follow up period.

One rectal follow-up swab sample from one initially positive dog was positive again for ESBL-PE after 10 months. The three remaining dogs, which were initially positive for ESBL-PE at T0, tested negative throughout the entire follow up period (table 2).

Raw food diet

Out of the 38 dogs with HC, four dogs received a (partially or wholly) raw feed diet. Three of these dogs tested positive for ESBL-PE carriage.

Of the 34 dogs, which did not receive raw feed diet, one tested positive for ESBL-PE carriage. There was a significant difference regarding feeding of a raw food diet and carriage of ESBL-PE in dogs with HC ($p = 0.002$).

Two of the 12 healthy dogs received a partially raw feed diet and neither of them was positive for ESBL-PE. There was no significant difference in carriage of ESBL-PE in dogs with HC and healthy dogs receiving a raw food diet ($p = 0.2$).

Hospitalization

Of the 38 dogs with HC, 7 dogs were hospitalized (> 24 hours) within 1 month prior to inclusion in this study. One of these dogs tested positive for ESBL-PE carriage. There was no significant difference regarding hospitalization and carriage of ESBL-PE in dogs with HC ($p = 1$). None of the healthy dogs was hospitalized within 1 month prior to inclusion in this study. Therefore, no comparison with dogs with HC was possible.

Discussion

The main aim of this study was to investigate the carriage rate of MRSA, MRSP, ESBL-PE, CPE in dogs with HC compared with that of healthy dogs. Four dogs with HC (10.5%) tested positive for ESBL-PE. Although this was not statistically significant compared with the tested healthy dogs, the percentage of ESBL-PE carriage appears to be higher than that reported by a larger study with healthy dogs in our country (2.8%).[22] Carriage rates < 10% have been reported in Canada, United Kingdom, Switzerland, United States, Mexico and Algeria; carriage rates > 10 % have been reported in Portugal, Tunisia, France, Turkey and Kenya.[22-32] This shows that carriage of ESBL-PE in dogs varies according to the world region. The carriage rate of ESBL-PE in HC dogs reported here seems higher than the values reported for healthy dogs in middle Europe and seems closer to the values reported from countries like Portugal, Tunisia and France. This gives evidence that dogs with HC are at increased risk for carriage of ESBL-PE.

An association between glucocorticoid therapy and MRSP carriage has previously been described in dogs.[17] Hypercortisolemia induces a state of immunocompromise and increases the susceptibility to various pathogens.[15,16] In humans, the underlying cause of hypercortisolemia influences the risk for secondary infections. Patients developing hypercortisolemia from exogenous corticosteroids and patients with ectopic ACTH syndrome are more prone to developing infections compared to patients with endogenous hypercortisolism.[16,33,34] The cause is not completely clear; however, higher cortisol levels in the blood of patients treated with exogenous corticosteroids or suffering from ectopic ACTH syndrome than in the blood of patients with endogenous hypercortisolism have been discussed.[16,34]

One dog in this study with newly diagnosed HC had multiple skin lesions on its paws. One additional swab sample from the paws tested positive for MRSP and confirmed MRSP skin infection. It is important to distinguish between infection and carriage. Skin samples are known to have a higher MRSP prevalence than samples taken from the nose, pharynx and rectum.[35] The main goal of this study was to determine the carriage rate of MRSA, MRSP, ESBL-PE, CPE in dogs with HC. The sampled sites (nose, pharynx, rectum) chosen are typical sites used to investigate carriage of MRSA, MRSP, ESBL-PE, CPE. Further studies evaluating skin infection or skin contamination with these bacteria in dogs with HC and evaluating the state of immunocompromise are therefore needed to completely evaluate the risk these patients pose for transmission among species.

As described previously, this study found an association between feeding and carriage of ESBL-PE in dogs with HC.[18,19,36] A high prevalence of ESBL-PE in raw meat diets has been described.[37,38] Farm animals, especially chickens, have been found to be a reservoir for ESBL-PE.[39-41] It is suspected that the ingestion of raw meat contaminated with ESBL-PE results in the transfer of these bacteria to the animal. One dog carrying ESBL-PE at the time of inclusion and being fed raw food during the entire study period, was negative at the second sampling time point, but again positive at the third sampling time point. The most likely explanation for the negative culture results at the second sampling time point seems intermittent shedding. However, recarriage through contaminated raw food, other infected dogs or persons or contaminated surfaces in the household are other possible explanations.[42]

We did not find CPE carriage in our study. However, looking at the so far low prevalence of CPE in companion animals reported by studies in middle Europe and at the rather low number of dogs included in this study, this is not surprising.[4,43] Nevertheless, surveillance studies in companion animals are needed to supervise the spread of this hazardous pathogen.

There are several limitations of this study. The first is the small number of dogs included. Another limitation is the inclusion of mainly treated dogs, which seemed well controlled (cortisol concentrations within the treatment target range). Whether inclusion of more newly diagnosed dogs with uncontrolled HC would have led to different results is unknown. A further limitation is that the state of immunocompromise was not assessed. To the authors' knowledge, assessment of the state of immunocompromise in dogs with HC is lacking in the literature. Further studies are therefore needed to evaluate the immune status of dogs with HC before and during treatment, and to correlate these results with the risk of carriage or infection rate with MRSA, MRSP, ESBL-PE, CPE.

In conclusion, this study provides, to the best of our knowledge, the first information about the carriage rate of MRSP, MRSA, ESBL-PE and CP-CRE in dogs with HC. Overall, we showed that the prevalence of ESBL-PE in dogs with HC seems higher than so far reported in healthy dogs. Interestingly, raw meat feeding was significantly associated with carriage of ESBL-PE in dogs with HC. Further studies are needed comparing carriage and infections of MRSA, MRSP, ESBL-PE, CPE in dogs with HC and other diseases to evaluate the risk for MDR bacteria transmission between individuals.

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Table 1: Prevalence of MRSA, MRSP, ESBL-PE and CPE carriage at inclusion

	Dogs with HC	**Healthy dogs**
MRSP	0/38	0/12
MRSA	0/38	0/12
ESBL-PE	4/38	0/12
CPE	0/38	0/12

HC, hypercortisolism; MRSP, methicillin resistant *Staphylococcus pseudintermedius*; MRSA, methicillin resistant *Staphylococcus aureus*; ESBL-PE, extended-spectrum β-lactamase-producing *Enterobacteriaceae*; CPE, carbapenemase-producing *Enterobacteriaceae*

Table 2. Carriage of MRSA, MRSP, ESBL-PE, CPE in dogs with HC at inclusion and during follow up

	Time T0	Time T1	Time T2	Time T3
MRSP	0/38	0/19	0/11	0/5
MRSA	0/38	0/19	0/11	0/5
ESBL-PE	4/38	0/19	1/11	0/5
CPE	0/38	0/19	0/11	0/5

HC, hypercortisolism; MRSP, methicillin resistant *Staphylococcus pseudintermedius*; MRSA, methicillin resistant *Staphylococcus aureus*; ESBL-PE, extended-spectrum β-lactamase-producing *Enterobacteriaceae*; CPE, carbapenemase-producing *Enterobacteriaceae*
T0: at inclusion; T1: 1.6-8.8 months after inclusion; T2: 4.3-10.8 months after inclusion; T3: 9.6-15.6 months after inclusion.

Danksagung

Diese Arbeit widme ich meiner Mama, die mich lehrt nach den Sternen zu greifen.

Während der Entstehung meiner Dissertation haben mich zahlreiche Personen meines beruflichen und privaten Umfelds begleitet und mich auf ganz unterschiedliche Art und Weise unterstützt. Hiermit möchte ich mich bei ein paar dieser Menschen ganz besonders bedanken.

An erster Stelle möchte ich meiner Doktormutter, Frau Prof. Dr. Nadja Sieber-Ruckstuhl, ganz herzlich dafür danken, dass Sie mir in dieser besonderen Zeit stets mit Rat und Tat zur Seite gestanden ist und mich immer sowohl bei der klinischen als auch wissenschaftlichen Arbeit unterstützt hat. Sie hat mir in zahlreichen Gesprächen immer wieder Anregungen und Hinweise gegeben, um diese prospektive Studie bestmöglich umzusetzen. Für ihre hilfsbereite und kompetente Betreuung danke ich ihr von ganzem Herzen.

Frau Dr. Wanda Burckhardt möchte ich vor allem für die Zeit zu Beginn meiner Doktorarbeit danken. Sie war sowohl bei der Ideenentstehung, als auch bei der Umsetzung dieser Arbeit beteiligt und ich danke auch Ihr für ihre herzliche und kompetente Betreuung in dieser Zeit.

Das Trio der Endokrinologie, Frau Prof. Dr. Claudia Reusch, Frau Prof. Dr. Felicitas Boretti und Frau Prof. Dr. Nadja Sieber-Ruckstuhl, hat mich von Anfang an sehr herzlich in das Team aufgenommen und mich schrittweise an die Arbeit in der Klinik herangeführt. Es war für mich eine intensive, spannende und lehrreiche Zeit, in der ich von den Besten lernen durfte und ich danke Ihnen für die zahlreichen Gespräche, die vielen aufschlussreichen Antworten auf meine Fragen und ihr Vertrauen in mich.

Frau PD Dr. Barbara Willi möchte ich für Ihren Rat auf dem Gebiet der multiresistenten Keime danken. Sie hat mir darüber hinaus ermöglicht, die Impfsprechstunde unter Ihrer Leitung durchzuführen und meine Kenntnisse im Zuge der klinischen Tätigkeit in der Infektiologie zu erweitern.

Nicht zuletzt danke ich meinen Freunden und meiner gesamten Familie, meiner verstorbenen Großtante Hildegart, die mir eine einmalige Zeit in Zürich geschenkt hat, meiner Mutter, meinen zwei Schwestern und meinem Ehemann, die mich in allen Lebenslagen immerzu liebevoll unterstützen. Sie sind mein zu Hause und das, auf was es im Leben ankommt.

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