



## Coryneform bacteria associated with canine otitis externa

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### ABSTRACT

This study aims to investigate the occurrence of coryneform bacteria in canine otitis externa. A combined case series and case–control study was carried out to improve the current knowledge on frequency and clinical significance of coryneform bacteria in samples from canine otitis externa. A total of 16 cases of otitis externa with involvement of coryneform bacteria were recorded at two referral veterinary hospitals in Denmark and the US, respectively. Coryneform bacteria were identified by partial 16S rRNA gene sequencing. *Corynebacterium auriscanis* was the most common coryneform species (10 cases). Small colony variants of this species were also observed. Other coryneform isolates were identified as *Corynebacterium amycolatum* (3 cases), *Corynebacterium freneyi* (2 cases) and an *Arcanobacterium*-like species (1 case). The coryneform bacteria were in all cases isolated together with other bacteria, mainly *Staphylococcus pseudintermedius* alone ( $n=5$ ) or in combination with *Malassezia pachydermatis* ( $n=5$ ). Some coryneform isolates displayed resistance to fusidic acid or enrofloxacin, two antimicrobial agents commonly used for the treatment of otitis externa in dogs. The frequency of isolation of coryneform bacteria was 16% among 55 cases of canine otitis externa examined at the Danish hospital during 2007. In contrast, detectable levels of coryneform bacteria were not demonstrated in samples from the acoustic meatus of 35 dogs with apparently healthy ears, attending the hospital during the same year. On basis of the current knowledge, these coryneform bacteria should be regarded as potential secondary pathogens able to proliferate in the environment of an inflamed ear canal.

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### 1. Introduction

Otitis externa in dogs is a disease of multifactorial etiology (August, 1988), in most cases associated with the presence of microorganisms. The occurrence and concentration of microorganisms in samples from canine otitis notably exceed those in clinically normal ears (Ginel et al., 2002), and some bacteria such as *Pseudomonas* and *Proteus* are only detected in inflamed ears (Yoshida et al., 2002;

Yamashita et al., 2005). *Malassezia pachydermatis*, *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Streptococcus canis* are among those often reported to be associated with canine otitis externa (Krogh et al., 1975; Kowalski, 1988; Bornand, 1992; Muller and Heusinger, 1994; Blanco et al., 1996; Breitwieser, 1997; Cole et al., 1998; Yamashita et al., 2005; Lyskova et al., 2007). Coryneform bacteria are regarded as part of the normal flora of the skin in dogs (Grono and Frost, 1969; Angus, 2004). They have been only sporadically reported in cases of canine otitis (Grono and Frost, 1969; Kowalski, 1988; Guedeja-Marron et al., 1998; Graham-Mize and Rosser, 2004; Zdovc, 2004). Little is known about species,

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frequency and phenotypic properties of coryneform bacteria isolated from clinical samples from dogs. The present manuscript describes a case–control study performed in Denmark to determine the frequency of coryneform bacteria in the ears of dogs with and without clinical signs of otitis externa. In addition, laboratory findings relative to 16 cases of otitis externa with occurrence of coryneform bacteria, recorded by two referral veterinary hospitals in Denmark and the US, respectively, are presented and discussed with focus on the clinical significance as well as the diagnostic and therapeutic implications associated with the occurrence of these bacteria in clinical specimens originating from dogs affected with otitis externa.

## 2. Materials and methods

### 2.1. Case–control study

A case–control study was performed at the Faculty of Life Science, University of Copenhagen. During 2007, ear swabs were obtained from 90 dogs attending the small animal hospital at this faculty, including 55 dogs with clinical signs of otitis externa (case group) and 35 dogs without clinical evidence of otitis externa (control group). Cotton swabs with Stuart medium (BBL Culture Swab, Becton Dickinson France, Le Pont de Claix, France) were rolled deep in the external ear canal. The swabs were cultured on Blood Agar prepared from Blood Agar Base (Oxoid, Basingstoke, Hampshire, England) containing 5% sterile bovine blood, and on Sabouraud Agar (Oxoid) supplied with chloramphenicol (25 µg/ml) for selective isolation of *Malassezia*. The non-coryneform isolates were characterized according to standard phenotypic methods (Barrow and Feltham, 1993). Isolates of Gram positive rods, preliminarily considered to be coryneform bacteria, were subjected to further genotypic and phenotypic characterization.

### 2.2. Case series study

Data were collected from a total of 16 cases of otitis externa associated with coryneform bacteria. Nine cases were recruited from the case–control study in Denmark. Seven additional cases were presented to the small animal referral hospital at the College of Veterinary Medicine, University of Tennessee, USA.

### 2.3. Genotypic and phenotypic identification of coryneform bacteria

Coryneform isolates obtained from the 16 cases under study were identified by partial 16S rRNA gene sequencing. DNA was extracted by boiling bacterial suspensions in distilled water for 20 min. PCR was conducted using universal eubacterial primers 37F (5'-GGC TCA GRW YGA ACG C-3') and 519R (5'-GTR TTA CCG CGG CTG CTG-3'). PCR products were purified using the PCR clean-up gel extraction kit (Nucleospin<sup>®</sup> Extract II, Machery-Nagel) and submitted to Macrogen Inc. (Seoul, South Korea) for sequencing on a DNA Analyzer 3730xl (Applied Biosys-

tems). BLAST search (Altschul et al., 1997) was performed using GenBank (Benson et al., 2006). The isolates were characterized biochemically by the API-Coryne system (BioMerieux, Inc., Marcy-l'Etoile, France) according to the manufacturer's instructions. Enzyme reactions were read after 24 h of incubation at 37 °C and acid production from carbohydrates was recorded after 48 h. Ability to hydrolyse tween 80 was examined by observation for opacity around colonies on Tween Agar (Blood Agar Base supplied with 1% (v/v) tween 80, 1% (v/v) of CaCl<sub>2</sub> solution containing 10 g per 100 ml and Tween Serum Agar (Tween Agar supplied with 1% sterile bovine serum). Ability to reduce tellurite was examined by observation of black colour of colonies on Tellurite Agar (Blood Agar Base supplied with 0.01% potassium tellurite) and Tween Tellurite Agar (combined Tween Agar and Tellurite Agar).

### 2.4. Antimicrobial susceptibility testing

The disk diffusion method was performed on Mueller Hinton Agar according to CLSI (Clinical and Laboratory Standards Institute, 2002). The following antimicrobial disks (Oxoid) were used: ampicillin (10 mg), enrofloxacin (5 mg), erythromycin (15 mg), fusidic acid (10 mg), gentamicin (10 mg), nalidixic acid (10 mg), tetracycline (30 mg), and trimethoprim/sulfamethoxazole (1.25 + 23.75 mg). Since no clinical breakpoints are available for corynebacteria of canine origin, resistance was defined on the basis of epidemiological cut-off values identified by analysis of the distribution of inhibition zone diameters. The minimum inhibitory concentrations (MICs) of enrofloxacin were determined by *E*-test according to the manufacturer's instructions (AB BIODISK, Sweden).

## 3. Results

### 3.1. Case–control study

From the 55 otitis cases monocultures were obtained from 23, consisting of *P. aeruginosa* ( $n = 13$ ), *S. pseudintermedius* ( $n = 4$ ), *M. pachydermatis* ( $n = 4$ ) or *Proteus mirabilis* ( $n = 2$ ). Mixed cultures were obtained from 23 other cases. The major combinations were *S. pseudintermedius* plus *M. pachydermatis* ( $n = 7$ ), *S. pseudintermedius* plus coryneform bacteria ( $n = 3$ ), and *S. pseudintermedius* plus *M. pachydermatis* plus coryneform bacteria ( $n = 3$ ). Other combinations were observed in 10 cases. Nine of the 55 otitis cases were culture negative. Coryneform bacteria were isolated from 9 of the 55 case dogs (16%). The coryneform bacteria were in all cases present together with other microorganisms such as *S. pseudintermedius* and *M. pachydermatis*. Coryneform bacteria were not detected in any of the 35 control dogs.

### 3.2. Case series study

The 16 cases of otitis externa from which coryneform bacteria were isolated are presented in Table 1. Most dogs (11/16) were older than 5 years. Seven cases (44%) were recorded as recurrent infections. The typical clinical presentation was chronic otitis externa with presence of yellow-brownish exudate, cerumen and erythema of the

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