First reported isolation of *Neisseria canis* from a deep facial wound infection in a dog

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Abstract

*Neisseria canis* was isolated in pure culture from a mandibular abscess in a dog. Ultrasound guided fine needle aspiration was used to obtain a sample from the abscess. Conventional bacteriological examination techniques followed by 16S rRNA gene sequencing from pure subculture and construction of a phylogenetic tree verified the isolate as *N. canis*. The 16S rRNA sequence analysis revealed that a broader phylogenetic platform is needed in the part of the phylogenetic tree where the canine pathogenic *N. canis* isolate is located. The canine pathogenic isolate was found to be resistant to cephalexin and trimethoprim.

Keywords: *Neisseria canis*, dog, wound infection, 16S rRNA
Case report. *Neisseria canis* has been shown previously to be a commensal of the oral cavity, saliva, dental plaque, muzzle, nasopharynx, and throat (1, 2, and 3) of dogs and cats. Several studies connected dog (1) and cat (5, 6) bites to *N. canis* infections in humans. To our knowledge this is the first reported case of a canine mandibular abscess containing a monoculture of *N. canis*.

A 2-year-old, male, Nova Scotia duck tolling retriever was presented with focal swelling on the left mandible together with pain when rotation and hinge action of the temporomandibular joint lasting for a few weeks. The animal was otherwise in a good general condition with normal vital parameters. The patient underwent anti-inflammatory treatment with carprofen (Rimadyl®), and antibiotics (cephalexin 25 mg/kg per os, q 24 h x 5 days). The dog was referred to the Norwegian School of Veterinary Science (NSVS), Small Animal Clinic for a computed tomography (CT) scan of the skull. The CT examination was performed under sedation using 15 µg/kg medetomidin and 0.1 mg/kg butorphanol intravenously. A rounded hypodense mass, about 2.5 cm in diameter caudolateral to the left angulus mandibulae was observed on CT scan. After intravenous application of the positive contrast medium (40 ml Iohexol, 300 mg I/ml Omnipaque®) a ring enhancement was observed around the hypodense centre (Figure 1). Further there was an ill-defined area of contrast uptake pooling between the mass, going ventrally around the mandible, between medial and lateral pterygoid muscle reaching to the oropharynx (Figure 2a). This tract could have been formed by a foreign body perforating from the oropharynx and reaching the lateral side of the mandible.

An ultrasound guided fine needle aspiration was performed after the CT scan (Figure 2b). The surrounding area was clipped and cleaned with iodine (Betadine®) and 70% ethanol was used to obtain an acoustic window for the ultrasound probe. The small abscess cavity was filled with anechoic contents and a few hyperechoic reflectors without distal shadowing were identified, surrounded by a thick tissue-like capsule. The small cavity was aspirated and 0.2
ml of a purulent-hemorrhagic material was obtained. Right away it was transferred to a sterile transport medium.

The sample arrived at the NSVS Routine Bacteriology and Mycology Diagnostic Services laboratory within 1 h after collection and immediately examined. Gram-negative polymorphonucleic cells were detected by direct examination after Gram staining. The sample was inoculated on two separate 5% sheep blood agar plates (Oxoid). One plate was incubated in 5% CO₂ atmosphere at 37°C for 24 h and the other plate was anaerobically incubated at 35°C for 48 h. In addition the sample was inoculated on a cystine lactose electrolyte-deficient medium (Brocalin agar; Merck, Darmstadt, Germany) plate at 35°C for 24 h to check the presence of lactose fermenting bacteria. After incubation aerobically in CO₂ enriched atmosphere, 50-100 yellowish, flat topped, coalescent colonies 2-3 mm in diameter without hemolytic activity were observed on blood agar. There was no growth on blood agar anaerobically or on Brocalin agar. After Gram staining of single colonies, Gram-negative cocci were observed, and the bacterial cells were approximately 2.5 to 3 µm in diameter. The isolate was catalase (strongly) and oxidase positive, but did not produce acid from glucose, maltose, fructose, sucrose, mannitol, mannose, lactose or saccharose and also did not produce indole. Also, nitrate reduction and gelatinase activity was detected however nitrite reduction and urease activity was absent using the API 20 NE system (BioMérieux, Marcy l’Etoile, France). According to the Bergey’s Manual of Determinative Bacteriology (8), the Gram negative cocci were identified as *N. canis*.

DNA was extracted from a loopful of pure subculture colonies incubated overnight using the DNeasy Blood & Tissue Kit® (Qiagen S.A., France). Following nucleic acid purification, the 5’ part of the 16S rRNA gene (corresponding to *Escherichia coli* positions 10 to 806) was amplified using primers V1 [5’-AGA GTT TGA TCA TGG CTC AGA-3’] and V3 [5’-GGT TAC CTT GTT ACG ACT TC-3’]. The cycling conditions included an initial denaturation for
3 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 56°C, and 2 min at 72°C; and a final extension for 10 min at 72°C. Two micro liters of the DNA extract was used for amplification in a total volume of 25 µl containing 2.5 µl 10x PCR Buffer, 1 µl 50 mM MgCl₂, 1 µl 10 mM dNTP mix, 5 pMol each of forward and reverse primers, 1U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania) and 16.4 µl sterile nuclease-free PCR grade water. DNA amplifications were performed in a GeneAmp 9700 PCR system thermocycler (Applied Biosystems, Foster City, USA). Positive (Escherichia coli NVH 1067/03) and negative control (sterilized dH₂O) samples were included in all amplifications. The PCR products were analyzed on 1.5% agarose gel stained with SYBR Safe® DNA Gel Stain (Invitrogen). After electrophoresis at 100V for 60 min, DNA bands were visualized by a Gel Doc™ XR+ Imaging System (Bio-Rad, Hercules, CA). For sequence analysis, amplicons were purified using a QIAquick PCR Purification Kit® (Qiagen S.A., France) and sequenced by GATC Laboratories (Konstanz, Germany) with V1 and V3 primers. The determined sequence (1037 bp) yielded a similarity score of 96% with a N. canis ATCC 14687 type strain (GenBank: L06170.1) and 98% with a reference sequence (GenBank: AY426974.1) which was previously isolated from human wound after a dog bite by (W. Geissdoerfer, C. Schoerner and M. Roellinghoff, unpublished data). Furthermore, using the 16S rRNA gene sequences of the related bacteria, a phylogenetic tree was constructed as described by (9). Sequences of 16S rRNA from Moraxella strains that sometimes have been confused with Neisseria strains by conventional bacteriological techniques (7), were included in the phylogenetic tree to classify the strain. The result clearly shows that N. animolaris is the closest phylogenetic taxonomic neighbour to the clinical canine isolate based on the 16S rDNA sequenced in this case (Figure 3), confirming the strain as belonging to the genus Neisseria. The phenotypical test results localize the canine pathogenic isolate from the abscess of this study to the species N. canis. However, the phylogenetic tree based on 16S rRNA sequences reveals that gene sequences
from more than the 16S rRNA gene are needed to create reliable taxonomic conclusions based on parallel phenotypic and genotypic diagnostic approaches.

Susceptibility against 11 selected antimicrobial agents was tested using the disc-diffusion method (Neo-Sensitabs, Rosco, Taastrup, Danimark). Inhibition zones were measured and interpreted according to the MIC break points of the Norwegian AFA group (2005, 2006), described in ‘User’s guide Neosensitabs®’ (http://www.rosco.dk). The isolate was found to be susceptible to amoxicillin-clavulanic acid, ampicillin, enrofloxacin, fucidin, lincomycin, neomycine, penicillin, spiramycin, tetracycline and trimethoprim-sulphonamide whereas it was resistant to cephalexin and trimethoprim.

*Staphylococcus intermedius*, *Proteus* spp., *Pseudomonas* spp. and *Escherichia coli* can be isolated from the major part of bacterial skin infections in dogs (11). In this case, empirically chosen broad spectrum treatment with cephalexin, a treatment commonly used for dog skin infections (4), failed. The patient was then subjected to systemic antimicrobial therapy with enrofloxacin (5 mg/kg per os, q 24 hours) according to the antimicrobial susceptibility profile. After three weeks of treatment, the patients pain when opening its mouth was completely resolved and focal soft tissue swelling on the mandible was completely gone.

The most frequently identified bacteria associated with the oral cavity of dogs are *Actinomyces* spp., *Streptococcus* spp. and *Granulicatella* spp. from saliva and *Porphyromonas* spp., *Actinomyces* spp. and *Neisseria* spp. from dental plaque (3). Due to the connection of *N. canis* with the oral cavity of dogs (1, 3), an abscess could be hypothesized to origin from a foreign body penetrating the oral mucosa. However, this was never verified and not demonstrated clearly in radiological findings. In such a situation with a wound, bacteria could penetrate the natural protective barriers of the oral mucosa and gain entry into the tissue below. An artificial tract made by a foreign body probably contaminated with the oral microbiota of the patient could result in a mixed infection. Bacteria of a mixed infection except *N. canis*
might have been killed by the initial treatment with cephalexin before sampling, explaining the pure culture of *N. canis* obtained upon culturing. The fact that a cephalexin resistant *N. canis* was able to continue the infectious process after 3 weeks of cephalexin treatment indicates strongly that *N. canis* from the oral microbiota of dogs can be able to sustain an infection in the soft tissues of its host as the sole pathogen.

Despite its reported opportunistic infection properties (6) and demonstrated pathogenicity (10) in human clinical infections, to our knowledge, a case of mandibular deep wound infection caused by *N. canis* in a natural canine host has not been reported earlier.


Figure 1. CT scan (A), post contrast CT scan in a soft tissue (B) and bone window (C).
Hypodense rounded structure lateral to the left angulus mandibulae can be identified with a ring enhancement and no bone involvement.
Figure 2a. The post contrast CT, 4 following slices (from cranial to caudal) showing focal contrast enhancement (white arrows) from the abscess to the oropharynx, ventral to the mandible and between the medial and lateral pterygoideus muscles.

Figure 2b. Transverse ultrasound image (GE Logiq 9, 15 MHz linear probe) of the anechoic cavities, containing a few hyperechoic reflectors (white arrow), surrounded with thick echoic capsule. Smooth hyperechoic contour of the mandible (grey arrow).
**Figure 3.** Phylogenetic neighbor-joining tree of the family *Neisseriasae* and *Moraxellaceae* based on nucleotide sequence of the 16S rRNA genes. *Pasteurella multocida* was used as an outgroup. ⬤, nodes with >95% bootstrap support for all analyses; ⬤, with >85% and ⬤, >75% bootstrap support. Values below 50% are not shown. Brackets define the gene accession numbers.