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The clinical significance of *Nicoletella semolina* in horses with respiratory disorders and a screening of the bacterial flora in the airways of horses

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ABSTRACT

Nicoletella semolina, a member of the family *Pasteurellaceae*, can be isolated from the airways of horses with respiratory disorders. However, its role as a potential or opportunistic pathogen is not clear nor is its presence as part of the normal flora. We therefore investigated the presence and bacterial load of *N. semolina* in healthy and diseased horses. Samples from a healthy control group were compared with samples from the routine analysis of horses with a clinical history of respiratory disorders. A total of 1770 nose swabs and 1132 tracheal aspirate samples were analysed and subjected to conventional bacteriological examination.

N. semolina was isolated from 12 (6%) of 207 nose samples from the healthy control group and from 42 (3%) of 1563 samples from horses with respiratory disorders. In tracheal aspirate, *N. semolina* was isolated from 7 (3%) of 211 samples from the control group and 49 (5%) of 921 samples from horses with respiratory disorders. Other bacteria were also isolated in laboratory analyses, the most commonly isolated bacterium in both the control group and the respiratory disorders group being *Streptococcus equi* subsp. *zooepidemicus*. It was isolated in 21% of tracheal aspirate from the control group and 33% of those from horses with respiratory disorders.

In conclusion, *N. semolina* is not a primary pathogenic bacterium, as it was isolated at similar frequencies in horses with respiratory disorders and those in the healthy control group.

1. Introduction

Equine infectious upper respiratory disease is a common and frequently recurring problem in horses worldwide. During recent five years, a new member of the family *Pasteurellaceae*, *Nicoletella semolina* (Kuhnert et al., 2004), has been isolated from the respiratory tract of about 200 horses at the National Veterinary Institute in Sweden (SVA). The samples originated from horses of different ages and breeds with a clinical history of various degrees of respiratory disorders, such as coughing, dyspnoea, nasal discharge, increased respiratory effort and exercise intolerance (Hansson et al., 2006). Most members of the family *Pasteurellaceae* occur as opportunistic organisms (Olsen et al., 2005). As commensals, they can be found in the alimentary, respiratory and genital tracts of healthy animals. In a study by Sternberg (1998), *Actinobacillus equuli* subsp. *equuli* was isolated from the oral cavity of 37% of horses studied. The role of *Streptococcus equi* subsp. *equi* (*S. equi*) as a pathogen in equine respiratory tract infections has been well documented, whereas *S. equi* subsp. *zooepidemicus* (*S. zooepidemicus*) is considered to be a microorganism that opportunistically causes disease (Dixon et al., 1995; Burell et al., 1996; Christley et al., 2001; Timoney, 2004). However, the role of *N. semolina* as a potential pathogen

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has not been investigated so far. The aims of this study were therefore to determine whether *N. semolina* is an emerging pathogenic species, opportunist or just part of the normal bacteriological flora. The study examined samples from a healthy control group without symptoms of respiratory disease and compared these with samples from horses with a clinical history of respiratory disorders identified in routine analysis at SVA.

2. Materials and methods

2.1. Horses

A total of 1770 nose swabs were collected for bacteriological examination in the period June 2009–October 2010, 207 from the healthy control group and 1564 from routine analysis of horses with different signs of respiratory disorder. Tracheal aspirate was collected from 1132 horses, 211 from the control group and 921 from the horses with diverse signs of respiratory disorders. The control horses were chosen among horses coming to the clinic for reasons other than respiratory disease. Most of the horses in the control group were sampled both by nose swabbing and tracheal aspiration, whereas horses with clinical disorders were mostly only sampled by nose swab or tracheal aspirate, the choice of between nose and tracheal samples among horses with clinical disorders were chosen by the clinical veterinarian depending of clinical signs and access to equipment. Horses in the control group received detailed clinical and respiratory examination and airway endoscopy when collecting the tracheal aspirate. The control group of healthy horses was initially represented by 214 nose swab samples and 218 tracheal aspirate samples, but 14 of these samples were withdrawn due to 7 horses showing signs of current airway inflammation and/or infection on clinical investigation (nasal discharge, abnormal breath sound, coughing or increased respiratory effort). The horses included in the study were of different breeds. Most of the horses in the healthy control group (54%) were standardbred trotters, 21% were ponies, 14% Swedish Warmblood, and the remaining 11% were of different breeds. In the group of horses with respiratory disorders, 30% were standardbred trotters, 32% Swedish Warmblood, 15% ponies and the remaining 23% were of different breeds. The age of the horses in the healthy control group varied between 1 and 23 years (median age 5 years), but most (58%) were youngsters aged between 2 and 5 years. In the group of horses with respiratory disorders, young horses also dominated. However, data were lacking on too many horses for calculation of mean age to be relevant. Foals (<1 year) were excluded in the study as foals were not sampled in the healthy control group.

2.2. Sample collection

Monolateral nose swab samples were collected in Aimes transport medium with charcoal (Copan, Brescia, Italy) after cleaning of the nostrils with cotton and isotonic saline solution. Tracheal aspirate was collected by an endoscopically guided catheter using 20 ml sterile 0.9% isotonic saline solution. Sampling of the healthy control group was approved by the Ethical Committee for Animal Experiments and the Swedish Board of Agriculture (diary number C32/2009). The horse owners in the control group gave their informed written consent on entering the study.

2.3. Bacteriological culture and identification

The nose swabs and tracheal aspirate samples were plated onto two blood agar plates with 5% horse blood and bromcresol purple lactose agar. One of the horse blood agar was inoculated with a streak of *Staphylococcus aureus* subsp. *aureus* (*S. aureus*) across the plate and incubated in an atmosphere containing 5% CO_2, to provide the V-factor for growth enhancement of fastidious bacteria. The bacterial growth on the plates was classified as sparse, moderate or rich, using a conventional technique known as "streaking". Growth only in the first third of the plate was classified as sparse growth, growth in the first and second third of the plate was classified as moderate growth and growth in all thirds was classified as rich growth.

N. semolina colonies were identified by characteristics such as grey, odourless, waxy colonies which could be moved around the agar plate without losing their shape; the colonies were not adherent and showed no haemolysis on horseblood agar plates; and the colonies produced catalase, oxidase and urease, but not esculinase. The organism was identified as a Gram-negative, pleomorphic, non-motile rod that was unable to produce acid from: rhamnose, glucose, lactose, maltose, sucrose, arabinose, cellobiose, mannitol, salicin or trehalose.

2.4. Sequencing and phylogenetic analysis of 16S rRNA genes

PCR products of the 16SrRNA genes were used for cycle sequencing with fluorescently labelled terminators (Big Dye; Applied Biosystems, Foster City, CA, USA) as described by the manufacturer and with a set of sequencing primers developed for members of the phylum Proteobacteria (Båverud et al., 2006).

The 16S rRNA sequences determined in this work were aligned manually with prealigned sequences retrieved from the Ribosomal Database Project II (Cole et al., 2008) and phylogenetic trees were constructed by neighbour-joining (Saitou and Nei, 1987). The 16S rRNA sequences of *N. semolina* have been deposited in GenBank under accession numbers JN036655–JN036696.

2.5. Statistical analysis

The data from the study were compiled and analysed using Microsoft Access and StatView software. Differences in prevalence of isolation of bacteria between the healthy control group and horses with respiratory disorders were examined by the chi-square test and were deemed significant at $p < 0.05$.

3. Results

3.1. Analysis of nose swabs and tracheal aspirate

N. semolina was isolated from 12 (6%) of the 207 nose swabs from the healthy control group and in 42 (3%) of the