Journal of Equine Veterinary Science 33 (2013) 561-564

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

Isolation of *Nicoletella semolina* from Equine Tracheal Washes

Karine Maillard DVM, MS [a], Eric A. Richard DVM, PhD [a], Peter Kuhnert PhD [c], Guillaume Fortier DVM, PhD [a,b], Albertine Léon PhD [a], Pierre-Hugues Pitel DVM, PhD [a]

[a] *Department of Animal Health, Frank Duncombe Laboratory, Caen Cedex, France*
[b] *University of Caen Basse-Normandie, SF4206 ICORE, EA4655 U2RM, Caen, France*
[c] *Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*

ARTICLE INFO

Article history:
Received 14 May 2012
Received in revised form
2 August 2012
Accepted 7 August 2012
Available online 13 September 2012

Keywords:
Nicoletella semolina
Horse
Cough
Tracheal wash
Airway inflammation

ABSTRACT

The aim of the present study was to describe the prevalence of *Nicoletella semolina* in equine airways and its relationships with cytological evaluation of tracheal wash (TW). Samples were collected in the framework of routine bacteriological diagnostics of equine TW between May 2010 and June 2011. *N semolina* has been isolated, along with either common pathogens or contaminants, from 19 (1.8%) of the 1,054 TW samples. Median TW neutrophil counts (87.0%) in specimens from *N semolina*-positive horses were significantly different from those from *N semolina*-"negative" horses (52.0%). The data presented in this report may suggest considering such bacteria in horses clinically suffering from airway inflammation.

1. Introduction

Bacteriological investigation of the tracheal wash (TW) is a common diagnostic tool in equine medicine. However, because any microbes detected usually belong to the normal flora of the upper airways, isolation of bacteria in TW may represent infection, transient lower airway colonization, or contamination of the sample. When investigating respiratory fluids from racehorses referred for poor athletic performance, bacterial respiratory infections, due to *Streptococcus zooepidemicus* and/or *Actinobacillus equuli*, ranged from 12.1% to 15.8% [1,2]. In a longitudinal study on inflammatory airway disease in Thoroughbred racehorses, the overall prevalence of *S zooepidemicus* and *Actinobacillus/Pasteurella* spp. was 30.4% and 27.3%, respectively [3]. The odds of suffering from inflammatory airway disease significantly increased with the numbers of these bacteria [4], whereas in another study, numbers more than 10^3 colony-forming units (cfu)/mL were significantly associated with coughing [5].

However, the methods being used in these studies were insufficiently discriminatory to identify the isolates to species level. On the other hand, a bacterium repeatedly isolated from horses with airway diseases, phenotypically and phylogenetically distinct from the other members of the family *Pasteurellaceae*, has previously been characterized and classified as *Nicoletella semolina* [6]. The aim of the present study was then to describe the prevalence of *N semolina* in equine respiratory tract and provide data concerning its possible implication in airway inflammation. In this manner, presence of *N semolina* was investigated in a cross-sectional study, as was its relationships with cytological evaluation of TW.

2. Materials and Methods

Samples included in the present study were collected in the framework of routine bacteriological diagnostics of

Corresponding author at: Eric A. Richard, DVM, PhD, Department of Animal Health, Frank Duncombe Laboratory, 1 Route de Rosel, 14053 Caen Cedex 4, France.
E-mail address: eric.richard@calvados.fr (E.A. Richard).

0737-0806/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2012.08.002

equine TW between May 2010 and June 2011. The TW samples were harvested by practitioners in the field either by percutaneous transtracheal aspiration or through an endoscope, transported by conventional mail, and processed within 24-48 hours from the time of sampling. Samples were centrifuged (2,500*g*, 10 minutes), and the pellet resuspended in 2 mL of the initial TW. Clinical signs noted by the veterinary practitioners have retrospectively been recorded for each case with *N semolina* isolation in TW. However, these data were not systematically available for the routine investigations performed in the laboratory.

Dilution series were done from liquid medium on agar plates, which were incubated for at least 24 hours (Table 1), and cultures were considered positive when bacterial concentrations reached 10^4 cfu/mL or more. The colonies were characterized by Gram staining, catalase, oxidase, glucose fermentation, and motility tests. Identification to species level was done by API system (Biomérieux, Marcy l'Étoile, France). According to previously published data [7], the different bacteria being isolated were classified as common possible pathogens (*S zooepidemicus*, *A equuli* subsp. *haemolyticus*, *Rhodococcus equi*, *Escherichia coli*, and *Stenotrophomonas maltophilia*) and probable contaminants (*Pseudomonas fluorescens*, *Pseudomonas putida*, *Moraxella* spp., and *Psychrobacter* sp.) of the equine respiratory tract. Initial phenotypic identification of *N semolina* isolates was achieved by culture (chocolate agar, 7% CO_2) and API NH test strips and then subsequently confirmed by molecular identification using partial sequencing of 16S rRNA and rpoB genes, known to be suitable for this purpose [6,8].

Cytological evaluation, including staining (May Grünwald Giemsa) and differential cell counts, (300 leukocytes) was systematically performed according to previously described procedures [2]. A nonparametric Mann–Whitney test was performed for comparisons of cytological profiles between groups of horses (*N semolina*-"negative" vs. -"positive"). Values of $P < .05$ were considered significant.

3. Results

N semolina has been isolated from 19 (1.8%) of the 1,054 TW samples (Table 2), with a median number of 10^6 cfu/mL (range: 10^5-10^7 cfu/mL). Other common pathogens were concomitantly found in 14 of 19 TW samples, among which 78.6% (11/14) revealed *S zooepidemicus* isolates, with numbers ranging from 9×10^5 to 9×10^7 cfu/mL (median: 6×10^6 cfu/mL). In the other five cases, *N semolina* was identified along with other probable contaminants. A history of cough and nasal discharge was present in 84.2% and 68.4% of cases, respectively, with *N semolina* detection. Cytological evaluation has been performed on 11 of the 19 TW samples, with neutrophil counts ranging from 6% to 97% (median: 87.0%; first quartile: 76.5%, third quartile: 89.5%). Cytology was also available for 790 of the 1,035 *N semolina*-"negative" specimens, with neutrophil counts ranging from 2% to 99% (median: 52.0%; first quartile: 17.5%, third quartile: 88.0%). TW neutrophil percentages were furthermore significantly different ($P = .048$) among horse groups (*N semolina*-"negative" vs. -"positive").

4. Discussion

This is, to our knowledge, the first published study describing the isolation of *N semolina* in equine TW samples, in conjunction with both clinical and cytological findings. The overall prevalence was slightly lower than in a recent report [9] applying polymerase chain reaction, which might be explained by the different methodological procedures being used. *N semolina* has actually been detected by polymerase chain reaction only in TW samples for 3% of healthy control horses and 5% of horses with respiratory disease, the difference being not significant [9]. In the present study, *N semolina* was systematically isolated by conventional methods, and all strains have subsequently been confirmed by 16S rRNA and rpoB gene sequencing. Some factors such as previous antibiotic therapy or bacteria numbers less than 10^4 cfu/mL may then have possibly limited the initial detection of this bacterium. Furthermore, the present study was conducted in a retrospective manner, with a TW bacteriological evaluation being the sole inclusion criterion. Such design may have introduced bias in the study, as the decision to submit a sample for bacterial culture rested with the attending veterinarian, and no specific control population has been identified in the cohort of samples. Another limitation of the study would be related to the sampling methodology used by the practitioners, especially the volume of fluid being instilled in the trachea and the procedure of TW (transtracheal aspiration or through an endoscope). However, minimal influence of the initial fluid volume on bacterial counts was anticipated, owing to the subsequent sample centrifugation and pellet resuspension being systematically performed at the laboratory. Furthermore,

Table 1
Synopsis of the standard conditions for inoculation and interpretation of the bacteriological analyses

Culture Medium	Provider	Temperature of Incubation	Incubation Duration	Atmosphere	Microorganism
Columbia agar with sheep blood	Oxoid[a]	37°C	48 hours	Anaerobic Aerobic	Strict anaerobic bacteria Strict aerobic bacteria Facultative aerobic bacteria
Mycoplasma liquid medium	Oxoid[a]	37°C	3 weeks	7% CO_2	*Mycoplasma* spp.
Chocolate agar	Biomerieux[b]	37°C	4 days	7% CO_2	*Bordetella bronchiseptica* *Nicoletella semolina*
Eosin–methylene blue agar	Oxoid[a]	37°C	48 hours	Aerobic	Enterobacteria *Pseudomonas* spp.
"*Rhodococcus*" agar	In-house	30°C	4 days	Aerobic	*Rhodococcus equi*

[a]Dardilly, France.
[b]Marcy l'Étoile, France.