Association of *Stenotrophomonas maltophilia* infection with lower airway disease in the horse: A retrospective case series

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**Abstract**

*Stenotrophomonas maltophilia* is being reported with increasing frequency as a human nosocomial pathogen, especially among immuno-compromised patients. To the authors’ knowledge, this pathogen has not previously been associated with lower airway disease in the horse. In this paper the clinical findings, laboratory diagnosis and response to treatment of seven cases of respiratory infection with *S. maltophilia* in horses, presented at three equine referral hospitals in Denmark in 2007, are described.

In all cases there was a clinical history of chronic coughing and abundant mucopurulent exudate was observed in the lower trachea on endoscopy. On culture of tracheal aspirate, grey, slow-growing colonies, identified as *S. maltophilia* by both API 20NE identification and 16s ribosomal DNA sequencing, were identified. All isolates had a similar antibiotic susceptibility pattern characterised by resistance to all penicillins and cephalosporins, and to imipenem, gentamicin, amikacin and rifampicin. Ribotyping and pulsed-field gel electrophoresis of the *S. maltophilia* isolates from different patients indicated that they were either indistinguishable or closely related. This study indicates that *S. maltophilia* can be associated with chronic lower airway disease in the horse and provides useful initial insights into the diagnosis, therapy and epidemiology of this novel condition.

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**Introduction**

*Stenotrophomonas maltophilia* is a Gram-negative, non-fermenting bacterium formerly known as *Pseudomonas maltophilia* and *Xanthomonas maltophilia*, but re-classified within the *Stenotrophomonas* genus since 1993 (Palleroni and Bradbury, 1993). This bacterium can be isolated from a wide range of environments, including freshwater, bottled water, sewage, soil, plants and fruits (Looney et al., 2009). In human medicine, *S. maltophilia* is recognised as a nosocomial pathogen, isolated primarily from immuno-compromised patients (Gales et al., 2001). Although occasionally considered a contaminant, or possibly a commensal, in clinical material (Enoch et al., 2007), it is generally accepted that this bacterium can behave as a true pathogen (Gales et al., 2001).

The most common and severe clinical manifestations of *S. maltophilia* infection in humans include bacteremia, endocarditis and respiratory tract disease (Denton and Kerr, 1998). Meningitis, in addition to urinary and gastrointestinal tract, skin and soft tissue and ocular infections are less common. Although generally regarded as a nosocomial pathogen, community-acquired *S. maltophilia* infection has been described (Gales et al., 2001). *Stenotrophomonas* bacteria are intrinsically resistant to treatment with β-lactams and are often also resistant to other antimicrobials. Antecedent therapy with carbapenems, extended-spectrum cephalosporins, aminoglycosides and fluoroquinolones are recognised risk factors for colonisation and infection (Denton and Kerr, 1998).

There is limited information relating to *S. maltophilia* infection in animals. Although isolated from fish and snakes (Hejnar et al., 2007), from bovine and ovine milk (Litopoulou-Tzanetaki and Vafopoulou-Mastrojannaki, 1995), from rabbit faeces (Draper et al., 1981), and from the gastrointestinal tract of laboratory animals (Toledo-Pereyra et al., 1974; Cloud-Hansen et al., 2007), *S. maltophilia* has only once been reported as an equine pathogen (Albini et al., 2009). However, one in vitro study of *S. maltophilia* and influenza concluded that co-infection with *S. maltophilia* could enhance the pathogenicity of equine influenza virus (Mancini et al., 2005).

In this study we describe the clinical and laboratory manifestations of *S. maltophilia* infection of the lower respiratory tract of seven horses as well as the response of these animals to antimicrobial therapy.
for >1 month and the animals had returned to their normal activities. Further clinical examinations continuing until signs of respiratory disease were absent.

The degree of mucus accumulation within the trachea was scored from endoscopic images as described by Gerber et al. (1988) and by Bacterial isolation and identification

Tracheal aspirate samples were transported to the laboratory by conventional methods and were cultured within 24–48 h on bovine blood agar incubated at 37 °C for up to 48 h. Cultures were considered positive when bacterial concentrations reached ≥10^5 CFU (colony forming units)/mL. The colonies were characterised by Gram-staining and by the oxidase, glucose fermentation and motility tests (Angen et al., 1997). Identification to species level was by API 20 NE (BioMérieux, France) and by 16S ribosomal DNA sequencing. The API 20 NE profiles were interpreted by Apiweb software (12). The 16S rRNA region was amplified by PCR (Guardabassi and Dalgaard, 2004) and sequencing was performed at Macrogen Inc. (Seoul) using Big-Dye (Applied Biosystems) on a 3730xl DNA analyser (Applied Biosystems). Sequences 211 bp in length (from position 92 to 303 according to E. coli numbering) were aligned by ClustalX 2.0 and further analysed by Basic Local Alignment Search Tool (BLAST) for species identification.

Materials and methods

Case description

In 2007, samples were obtained from two foals (3–4 months old) and five adult horses (7–18 years old) with a history of lower airway disease as part of a survey of antibiotic resistance (Table 1) (Winther et al., 2008). The seven animals were presented at the three largest equine referral hospitals in Denmark: Højgaard Equine Hospital (Hospital A), The University Hospital for Large Animals at The University of Copenhagen (Hospital B) and Ansager Equine Hospital (Hospital C).

All horses were clinically examined and this included an endoscopic examination of the respiratory tract. The degree of mucus accumulation within the trachea was scored from endoscopic images as described by Gerber et al. (1988). Tracheal aspirates were collected without a guarded catheter and were transported to the laboratory on the day of collection. Endoscopes were regularly swabbed after disinfection to ensure that cross-contamination of samples was not occurring. Horses were treated with antimicrobials in accordance with antimicrobial susceptibility testing and were discharged with weekly follow-up clinical examinations continuing until signs of respiratory disease were absent for >1 month and the animals had returned to their normal activities. Further examinations with sampling of tracheal aspirate continued for a period of 4 months.

To isolate and identify the bacterial isolates, tracheal aspirate samples were collected and transported to the laboratory where they were subcultured onto blood agar and MacConkey agar plates. The isolates were identified by Gram-staining and by the oxidase, glucose fermentation and motility tests (Angen et al., 1997). Identification to species level was by API 20 NE (BioMérieux, France) and by 16S ribosomal DNA sequencing. The API 20 NE profiles were interpreted by Apiweb software (12). The 16S rRNA region was amplified by PCR (Guardabassi and Dalgaard, 2004) and sequencing was performed at Macrogen Inc. (Seoul) using Big-Dye (Applied Biosystems) on a 3730xl DNA analyser (Applied Biosystems). Sequences 211 bp in length (from position 92 to 303 according to E. coli numbering) were aligned by ClustalX 2.0 and further analysed by Basic Local Alignment Search Tool (BLAST) for species identification.

Table 1

<table>
<thead>
<tr>
<th>Case number</th>
<th>Signalment</th>
<th>History</th>
<th>Clinical findings</th>
<th>Bacteriological findings</th>
<th>Treatment and outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oldenburg horse,</td>
<td>Coughing for 2 weeks.</td>
<td>Mucopurulent nasal discharge, tachypnoea and generalised wheezing on lung auscultation.</td>
<td>Mixed flora of Stenotrophomonas maltophilia (isolate 1a) and Enterobacter agglomerans.</td>
<td>TMS® for 10 days plus prednisolone and clenbuterol. Response to treatment but then relapsed.</td>
</tr>
<tr>
<td></td>
<td>7 year old, male</td>
<td>No previous treatment.</td>
<td>Mucus accumulation score of 4.5.</td>
<td>50% S. maltophilia (isolate No. 1b) and 50% Pseudomonas fluorescens.</td>
<td>Tetracycline for 7 days plus prednisolone, clenbuterol and mucolytics for 14 days. Recovered slowly over 4 months</td>
</tr>
<tr>
<td></td>
<td>Hospital A</td>
<td>2 months later – intermittent cough</td>
<td>Tachypnoea and generalised wheezing on lung auscultation.</td>
<td>Mucus accumulation score of 3</td>
<td>Tetracycline for 5 days. Responded well to treatment. Clinical normal 2 months later</td>
</tr>
<tr>
<td>2</td>
<td>Icelandic horse,</td>
<td>Coughing and depression for 3 weeks.</td>
<td>Tachypnoea, coughing, biphasic abdominal respiration.</td>
<td>Mucus accumulation score of 5</td>
<td>Unknown, as owner declined treatment and follow-up assessment</td>
</tr>
<tr>
<td></td>
<td>7 year old, female</td>
<td>No previous treatment.</td>
<td>Thick mucopurulent exudate on microscopic examination.</td>
<td>A mixed flora dominated by S. maltophilia (isolate 3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Danish Warmblood,</td>
<td>Coughing for 1 month.</td>
<td>Rattles in trachea, generalised wheezing on lung auscultation.</td>
<td>Mucus accumulation score of 4.6</td>
<td>Tetracycline for 10 days plus prednisolone and mucolytics – responded, but relapsed after 6 weeks. Doxycycline for 10 days – responded, but relapsed after 2 months. Further doxycycline for 14 days – responded well. Clinical normal 4 months after initial treatment</td>
</tr>
<tr>
<td></td>
<td>18 year old, male</td>
<td>No previous treatment.</td>
<td>Thick mucopurulent exudate on microscopic examination.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Danish Warmblood,</td>
<td>Coughing and nasal discharge for 4 months.</td>
<td>Tachypnoea generalised wheezing on lung auscultation.</td>
<td>Pure culture of S. maltophilia (isolate 4)</td>
<td>Tetracycline for 10 days plus prednisolone and mucolytics – responded, but relapsed after 6 weeks. Doxycycline for 10 days – responded, but relapsed after 2 months. Further doxycycline for 14 days – responded well. Clinical normal 4 months after initial treatment</td>
</tr>
<tr>
<td></td>
<td>7 year old, male</td>
<td>No previous treatment.</td>
<td>Acute respiratory distress-like syndrome.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital A</td>
<td></td>
<td>Thick mucopurulent exudate in trachea.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Danish Warmblood,</td>
<td>Coughing, poor weight gain and depression for 6 weeks.</td>
<td>Harsh lung sounds on auscultation. Mucopurulent exudate in trachea.</td>
<td>Sparse growth of S. maltophilia (isolate 5a)</td>
<td>Tetracycline for 8 days. Recovered and gained weight with no exudates in trachea. Clinical normal 2 months later</td>
</tr>
<tr>
<td></td>
<td>3 month old, male</td>
<td>Treated previously with TMS for 8 days</td>
<td>Mucus accumulation score of 4. Endoscopic examination 2 weeks later – almost no exudate.</td>
<td>Tracheal aspirate after treatment – mixed flora dominated by S. maltophilia (isolate 5b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital B</td>
<td>Broncho-pneumonia for 2 weeks. No response to penicillin</td>
<td>Mucus accumulation score of 0.7</td>
<td>Pure culture of S. maltophilia (isolate 6)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Trotter foal,</td>
<td></td>
<td>Tachypnoea, harsh lung sounds. Endoscopy not performed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 month old, male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Icelandic horse,</td>
<td>Coughing, tachypnoea, lethargy.</td>
<td>Harsh lung sounds, tachypnoea. Thick mucopurulent exudate in trachea.</td>
<td>Pure culture of S. maltophilia (isolate 7)</td>
<td>Tetracycline for 5 days plus mucolytics and prednisolone. Responded well to treatment. 3 months later relapsed with severe, chronic respiratory distress. Owner declined further treatment</td>
</tr>
<tr>
<td></td>
<td>16 year old, female</td>
<td>No response to TMS for 9 days</td>
<td>Endoscopic examination 5 days after treatment – significantly decreased amounts of exudate.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital A</td>
<td></td>
<td>Mucus accumulation score of 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Hospital A, Højgaard Equine Hospital; Hospital B, The University Hospital for Large Animals, University of Copenhagen; Hospital C, Ansager Equine Hospital.
b TMS = Trimethoprim-sulfadiazine.
c MIC = Minimum inhibitory concentration.
Antimicrobial susceptibility was measured by broth microdilution (Vizion System, TREK Diagnostic systems) using commercially prepared panels (Companion/Equine MIC Plate, Trek Diagnostic Systems). The plates were inoculated and interpreted according to Clinical and Laboratory Standards Institute standards (NCCLS, 2003).

Genotyping of isolates

A total of nine *S. maltophilia* isolates were genotyped by ribotyping and pulsed-field gel electrophoresis (PFGE). These included two isolates from horses that had relapsed after their first treatment. Ribotyping was performed as previously described (Christensen et al., 1993). In brief, bacterial cells from overnight cultures were lysed using 10% sodium dodecyl sulphate and the DNA was purified by phenol–chloroform extraction and precipitation with isopropanol. DNA fragments digested with EcoRI (Boehringer–Manhem) were separated overnight in 0.8% agarose gel and transferred to nylon membranes (Hybond N) by vacuum blotting. Following DNA fixation at 80°C for 60 min, the membranes were hybridised with a probe obtained from *Escherichia coli* 16s and 23s rRNA and labelled by reverse transcriptase (Boehringer–Manhem).

The PFGE was performed as described by Jumaa et al. (2006). Briefly, plugs were prepared using bacterial suspensions obtained from overnight cultures. These were lysed using lysis buffer at 37°C for 5 h and then incubated overnight at 56°C in proteinase K buffer. A 1 mm-thick segment from the plug was digested with XbaI (New England Biolabs) overnight at 37°C. Electrophoresis was performed on the CHEF-DR III System (Bio-Rad Laboratories) with 6 V/cm for 22 h at 14°C using an initial switch time of 5 s and a final switch time of 35 s with linear ramp. The gel was analysed using Gelcompar II (Applied Maths), and cluster analysis was performed by UPAGMA based on the Dice similarity coefficient, with optimisation and position tolerance set at 1%, respectively. Isolates showing 80% or higher homology were considered related or indistinguishable and assigned to the same PFGE type (A). Isolates showing minor band differences within the same PFGE type were considered distinct sub-types.

Results

Clinical history and findings

All horses had a history of chronic cough with or without nasal discharge. Coughing had been ongoing for between 1.5 and 4 months prior to referral. Three of the horses had been treated with antimicrobials (trimethoprim-sulfadiazine or penicillin) by the referring veterinarian. Different clinical manifestations of chronic respiratory disease were observed, including increased vesicular to harsh lung sounds, tachypnoea, nasal discharge, and occasionally dyspnoea (Table 1). Pyrexia was not a feature of any case at referral but had been a feature of the previous clinical history. A prominent finding in all animals was abundant, thick, mucopurulent exudate in the trachea, sometimes filling approximately one-third of the lumen (Fig. 1A and B). Mucus accumulation scoring of endoscopic images found a mean score of 4.3 (±1.1). The results of cytological examination of the aspirates were not recorded and no breed or sex predilection for infection was identified.

Bacterial isolation and identification

*S. maltophilia* was isolated from tracheal aspirates from all horses at concentrations of between 10⁴ and 10⁸ CFU/mL. Isolated colonies were typically small (<1 mm) after overnight incubation but enlarged and became grey in colour, in the area of confluent growth on 48 h incubation. Slender Gram-negative rods, either single or in pairs, were observed on microscopic examination. All isolates were catalase-positive, oxidase-negative, did not ferment glucose and were motile. Sequencing of 16s rDNA revealed two sequence types (1 and 2) differing by two point mutations at positions 137 (C→T) and 139 (G→T). Both sequences were 100% identical to reference *S. maltophilia* strains LMG 958-T (accession number X95923.1) and LMG 10874 (accession number AJ131783.1), respectively. The isolates displayed a typical API 20 NE profile (1-4-7-2-3-4-1) with a good identification score at species level (% ID = 99.9). In cases 1 and 5, *S. maltophilia* was re-iso-

Antimicrobial therapy

Three horses (cases 4, 5 and 7) were initially treated with oxytetracycline (Engemycin, Intervet) for 7–10 days (8 mg/kg once daily). Of the remaining 4 horses, 1 adult horse (case 2) was treated with trimethoprim-sulfadiazine (TMS) (Norodine, ScanVet Animal Health) for 5 days (30 mg/kg twice daily), another adult (case 1) was treated with TMS for 10 days, but on relapse was treated with oxytetracycline. A foal (case 6) responded to ampicillin (Pentrexyl, Bristol-Myers Squibb AB) at 20 mg/kg three times daily and gentamicin (Gentaject, Franklin Pharmaceuticals Ltd.) at 6.6 mg/kg once daily despite the fact that the in vitro susceptibility tests indicated resistance to both antibiotics. One horse (case 3) was not treated as the owner declined therapy.

Although all cases responded well to oxytetracycline, only two adults (cases 1 and 2) recovered over a 4 month period without relapse. Clinical relapses were observed in the other two adult horses...
Resistance was defined according to Clinical and Laboratory Standards Institute standards (NCCLS, 2003). Resistance was defined according to Clinical and Laboratory Standards Institute standards (NCCLS, 2003). Resistance was defined according to Clinical and Laboratory Standards Institute standards (NCCLS, 2003). Resistance was defined according to Clinical and Laboratory Standards Institute standards (NCCLS, 2003). Resistance was defined according to Clinical and Laboratory Standards Institute standards (NCCLS, 2003). Resistance was defined according to Clinical and Laboratory Standards Institute standards (NCCLS, 2003).

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Minimum inhibitory concentration (MIC) breakpoint (µg/mL)</th>
<th>MICa</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≤16/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≤8/32/32/32</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>≤8/32/32/32</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>≤6/8/8/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤6/8/8/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤4/8/8/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤6/16/16/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>≤6/16/16/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Penicillin</td>
<td>≤6/16/16/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Rifampin</td>
<td>≤6/16/16/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤6/16/16/16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Trimethoprim/Clavulanic acid</td>
<td>≤6/16/16/16</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

a MIC, minimum inhibitory concentration ranges are indicated for penicillin, rifampin and tetracycline since minor variations were observed among isolates. b NA resistance to ceftiofur resistance breakpoints are not available for this bacterial species. R, resistant; I, intermediate; S, susceptible.

(cases 4 and 7) as soon as treatment ceased. An additional treatment with oxytetracycline IV or doxycycline (Doxycycline, Biovet) orally (10 mg/kg twice daily) for 10–14 days was carried out in these animals. Both foals (cases 5 and 6) recovered with treatment and did not relapse. Additional treatment with bronchodilators, non-steroidal anti-inflammatory drugs, steroids and mucolytics were considered necessary for some of the horses (case 1, 4, 7), because of the clinical severity of the respiratory distress.

**Genotyping of isolates**

Ribotyping of the nine isolates identified two main types (I and II) with minor differences within each (data not shown). The five ribotype I isolates were all from animals attending Hospital A over a period of 10 months. Ribotype I S. maltophilia isolates were also cultured from cases 1 and 5 during follow-up sampling. The two ribotypes II isolates were recovered from horses that had attended different hospitals (A and C).

PFGE demonstrated better discriminatory power than ribotyping in differentiating isolates. Five PFGE types were identified and indistinguishable (A1) or closely related sub-types (A2 and A3) were found in horses attending Hospital A (Fig. 2). In case 1, the isolate obtained during follow-up sampling was also an A1 sub-type. In contrast, the two isolates from case 5 were unrelated (types B and C) (Fig. 2).

**Discussion**

In this study, S. maltophilia was isolated from seven horses with clinical signs of lower airway disease and with mucopurulent exudate in the trachea. In three cases (4, 6 and 7), S. maltophilia was isolated in pure culture and was the dominant colony type in two further cases (3 and 5). These data suggest this bacterial species can act as a respiratory pathogen in horses, as also recently reported by Albini et al. (2009), and as reported in immunocompromised human patients (Gales et al., 2001).

PFGE demonstrated distinct S. maltophilia sub-types among these equine isolates, with two different sub-types associated with case 5. Isolates from human patients where infection was acquired from hospital and community settings have been found to be highly heterogeneous (Hauben et al., 1999). According to published criteria for the interpretation of PFGE patterns (Tenover et al., 1995), bacterial isolates with 2–3 band differences should be classified as closely related. The fact that the isolates from three horses attending Hospital A were indistinguishable (sub-type A1) and the isolates from two additional horses were closely related (sub-types A2 and A3) suggested a possible nosocomial outbreak. Given that the horses attended the hospital on different days and did not have direct contact, it is possible that the bacteria were transmitted indirectly through contamination of the hospital environment or staff.

Outbreaks of nosocomial S. maltophilia infection in humans have been associated with the hospital environment including water, ice and re-usable medical devices (Dignani et al., 2003). In the future, it will also be important to consider if these equine S. maltophilia isolates have zoonotic potential.

In the current study all horses had a history of chronic coughing and the presence of abundant mucopurulent exudate in the trachea on endoscopy. Since animals initially responded well to treatment with oxytetracycline, it could be speculated that the clinical relapses that occurred reflected insufficient treatment periods. It is also possible however, that these relapses were a consequence of (as yet) unidentified factors. Underlying hepatobiliary, chronic pulmonary and cardiovascular diseases (Nicodemo and Paez, 2007) as well as organ transplantation, dialysis, intravenous drug use and HIV infection are associated with S. maltophilia infection in humans (Fujita et al., 1996; Hauben et al., 1999). Risk factors identified in human patients are, in order of decreasing risk, previous antimicrobial therapy, use of central venous catheters, neutropaenia, prolonged hospitalisation, admission to intensive care, mechanical ventilation or tracheotomy, haematological malignancy, and exposure to patients with S. maltophilia wound infections (Denton and Kerr, 1998).

**Table 2**

Details of the minimum inhibitory concentrations (MIC) of antimicrobials tested against equine isolates of Stenotrophomonas maltophilia by broth microdilution.

![Fig. 2. Dendrogram of pulsed-field gel electrophoresis (PFGE) profiles of equine Stenotrophomonas maltophilia isolates numbered as in Table 1. Five distinct PFGE types (A-E) were identified, including three related sub-types (A1, A2 and A3).]
The chronicity of the clinical signs in this study and the isolation of *S. maltophilia* from some of the horses in mixed cultures suggest this bacterium may act as a secondary opportunist as well as primary pathogen in this species. It is generally assumed that *S. maltophilia* has low pathogenicity and, in humans, infection is unusual in patients without underlying conditions (Johnson and Duckworth, 2008). *S. maltophilia* infection usually occurs in immunocompromised humans, mostly as hospital-acquired infections (Looney et al., 2009). The respiratory tract is the most common site for such acquired infections with incidence rates of 4% at some hospitals and such patients often have compromised lung function because of diseases such as cystic fibrosis (Gales et al., 2001).

Given that tracheal colonisation is enhanced by the presence of mucus (Gerber and Robinson, 2006), *S. maltophilia* can be considered both a primary pathogen and a secondary opportunist whose growth is favoured by the mucus produced as a result of pre-existing disease. Infection without disease may have occurred in case 6 in our study, where, despite resistance of *S. maltophilia* to the drugs used, the horse responded well to treatment. Another bacterial pathogen that was not detected may have been responsible for inducing disease in this case.

*S. maltophilia* is intrinsically resistant to β-lactams, aminoglycosides and fluoroquinolones (Looney et al., 2009). These features, except for fluoroquinolone, were noted in the isolates identified in this study. Resistance to β-lactams is mediated by production of at least two β-lactamases, one of which is resistant to β-lactamase inhibitors such as clavulanic acid and breaks down carbapenems, whereas aminoglycoside and quinolone resistance result from mutations in the genes encoding outer membrane proteins (Gilligan et al., 2003).

Potentiated sulfonamides are the drugs of choice against infections of humans with this organism but resistance to these drugs is reported as emerging during treatment (Ansari et al., 2007; Looney et al., 2009). Although none of the isolates in this study were ultimately found to be resistant to TMS, some were initially erroneously reported as resistant based on preliminary disc diffusion tests (data not shown). Since difficulties in interpretation of such tests on *S. maltophilia* isolates are well documented (Gilligan et al., 2003), all isolates were re-tested by quantitative minimum inhibitory concentration determination and were found to be susceptible (NCCLS, 2003). This technical problem did not have any negative impact on the clinical outcomes and tetracyclines appear to be effective in treating *S. maltophilia* respiratory infections in horses. Tetracyclines, especially minocycline and doxycycline, are often used in the treatment of severe *S. maltophilia* infections in humans, either alone or in combination with potentiated sulfonamides (Denton and Kerr, 1998).

Frequently, in veterinary clinical microbiology, identification of bacterial isolates is based on standard phenotypic tests that limit identification to the genus/group level and this is particularly true for Gram-negative non-fermentative bacteria. This study demonstrates that both commercial identification systems and 16S rRNA sequencing can be successfully employed to sub-type *S. maltophilia* or other bacterial species. Without such additional assessments, *S. maltophilia* could be erroneously reported as a non-specific *Pseudomonas* spp. As the cost and labour associated with such DNA sequencing decreases, clinical microbiology laboratories will have the opportunity to employ this technique in identifying isolates more rapidly and accurately.

Although the close association between abundant, pure growths of *S. maltophilia* with clinical respiratory disease in horses in this study provides strong evidence that this bacterium can act as a pathogen in this species, future cytological assessment of tracheal aspirate would provide further evidence of its significance and help elucidate the pathogenic role of the organism. Such assessment was not possible in the current study due to its retrospective nature.

Conclusions

This study provides evidence that *S. maltophilia* can be associated with chronic respiratory disease in the horse and provides useful initial insights into the diagnosis, therapy and epidemiology of this novel condition. Involvement of this organism should be suspected in equine patients with a history of respiratory signs and that present on endoscopy with abundant tracheal mucus. Our results indicate that *S. maltophilia* infection in horses could occur as nosocomial outbreaks. It is important to emphasise that the antibiotics most commonly used for treating equine respiratory infections are not active against *S. maltophilia* and that TMS is the drug combination of choice for infection in humans. The results of this study suggest the prolonged administration of tetracyclines (>10–14 days) is an effective treatment, especially in adult horses.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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