

## *Helcococcus ovis* Isolated from a Pulmonary Abscess in a Horse

Chantal M. Rothschild,<sup>1</sup> J. Lindsay Oaks,<sup>2,3</sup> Jennifer K. Schaupp,<sup>3</sup> Fred R. Rurangirwa,<sup>2,3</sup>  
Debra C. Sellon,<sup>1</sup> and Melissa T. Hines<sup>1\*</sup>

Departments of Veterinary Clinical Sciences<sup>1</sup> and Veterinary Microbiology and Pathology<sup>2</sup> and Washington  
Animal Disease Diagnostic Laboratory,<sup>3</sup> Washington State University, Pullman, Washington 99164

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***Helcococcus ovis*, a recently described organism cultured from sheep, was isolated in pure culture from a pulmonary abscess in a horse. This is the first report of this organism in horses and the first report in veterinary medicine to clearly demonstrate a pathogenic role for this organism.**

*Helcococcus* is a new genus of catalase-negative, facultatively anaerobic, gram-positive cocci that was first described in 1993 by Collins et al. (7). The prototype of this genus, *Helcococcus kunzii*, was originally isolated as part of mixed infections from wounds in the lower extremities of debilitated human patients and was regarded as a weakly pathogenic, opportunistic member of the human skin flora (3, 9, 12). More recently, however, two reports demonstrated the presence of *H. kunzii* in large numbers in an infected sebaceous cyst (10) and in a breast abscess (4) in patients without significant immunocompromise, suggesting that this bacterium could be a more virulent opportunistic pathogen.

In 1999, organisms similar to *H. kunzii* were isolated as part of mixed infections from sheep, in one case from milk and in another case from the lungs, liver, and spleen at necropsy (6). These isolates were phenotypically different from *H. kunzii*, and comparative 16S ribosomal DNA (rDNA) gene sequencing showed the sheep isolates to be approximately 4% divergent from *H. kunzii*. Therefore, it was proposed that the isolates be classified as a new species, *Helcococcus ovis* (6). In this report, we describe the isolation in pure culture of *H. ovis* from a pulmonary abscess in a horse, indicating that this organism has a wider host range and greater pathogenic potential than previously believed.

**Case report.** A 6-year-old quarter horse gelding with a history of recurring pleuropneumonia of 8 months' duration was presented to the Veterinary Teaching Hospital of Washington State University. Initially, the horse had suffered a penetrating wound in the ninth intercostal space on the right side of the thorax, and since then, intermittent coughing, dyspnea, tachypnea, mucopurulent nasal discharge, pyrexia, and pleurodynia had been evident. Antibiotic treatment instituted throughout this period by the referring veterinarian included procaine penicillin, gentamicin, and enrofloxacin, and after the intercostal space wound healed, chest tubes were occasionally placed for drainage of accumulated septic pleural fluid. By the fifth or sixth month, the patient seemed to have recovered from the condition. However, clinical signs recurred 1 week prior to presentation at the Veterinary Teaching Hospital of Washing-

ton State University, where thoracic radiographs revealed a large radiopaque fluid-filled mass which occupied approximately 65% of the area in the caudal dorsal aspect of the left lung. Thoracic ultrasonography revealed a consolidated, fluid-filled structure in the left hemithorax, extending from ribs 8 to 13 in width and from 10 cm ventral from the dorsal midline to the ventral aspect of the lung. The structure was consistent with a diagnosis of an abscess. The right lung was unaffected.

Samples obtained by thoracocentesis with aspiration of the abscess fluid and transtracheal wash were submitted for aerobic and anaerobic cultures, for sensitivity, and for cytology. The horse underwent a surgical partial-rib resection and aggressive drainage of purulent material, and necrotic tissue was removed. Subsequently, the patient was placed on oral trimethoprim-sulfadiazine (30 mg/kg of body weight every 24 h) for a period of 2 months and seemed to be recovering well.

**Bacteriology.** Gram stains of the thoracic exudate (abscess) showed occasional gram-positive cocci, many in pairs. Purulent material obtained by thoracocentesis and transtracheal wash was inoculated onto Trypticase soy agar–5% sheep blood with a streak of *Staphylococcus aureus* and onto chocolate agar for aerobic culture in a 5% CO<sub>2</sub>-enriched atmosphere at 35°C. Samples were also inoculated onto Columbia agar–5% sheep blood for anaerobic incubation at 35°C. An isolate was recovered in large numbers from both the thoracocentesis and transtracheal-wash samples and in pure culture from the thoracocentesis sample. The transtracheal-wash sample also contained a few colonies of *Pseudomonas* spp., which are common contaminants in equine transtracheal-wash samples and were not believed to be significant in this case. The predominant isolate was a facultatively anaerobic, catalase-negative, gram-positive coccus that formed clusters. The isolate grew aerobically on the chocolate and blood agars. Initially, the isolate grew only within the hemolysis zone of the *S. aureus* on the blood agar; however, after subculturing, it grew readily on nonhemolyzed blood agar. At 24 h, the colonies were pinpoint, nonpigmented, and nonhemolytic, but they were slightly alpha-hemolytic after 72 h. The isolate was lipophilic, showing markedly enhanced growth in brain heart infusion broth supplemented with 0.1% Tween 80. The isolate was biochemically characterized with the API 20 Strep kit (Biomérieux), which assigned it profile number 0440000. This biochemical profile indicated the production of β-glucuronidase and leucine arylamidase. The isolate did not produce acetoin, β-glucosidase, pyrrolidonyl aryl-

\* Corresponding author. Mailing address: Department of Veterinary Clinical Sciences, Washington State University, P.O. Box 647060, Pullman, WA 99164-7060. Phone: (509) 335-0711. Fax: (509) 335-3330. E-mail: mth@vetmed.wsu.edu.

amidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase, alkaline phosphatase, or arginine dehydrolase and did not hydrolyze hippurate. The isolate did not produce acid from ribose, L-arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, starch, or glycogen. This profile resulted in a good identification of the isolate as *Abiotrophia* (*Granulicatella*) *adiacens*. However, the lack of cocci in chains, the ability to grow on blood agar without staphylococcal hemolysins, and the incomplete database for the API 20 Strep kit made this identification questionable. Antimicrobial susceptibility testing using Etest strips (A-B Biodisk) determined that the isolate was susceptible to penicillin (MIC,  $<0.016$   $\mu\text{g/ml}$ ) and clindamycin (MIC,  $0.125$   $\mu\text{g/ml}$ ) and resistant to metronidazole (MIC,  $>256$   $\mu\text{g/ml}$ ).

To obtain a definitive identification of the isolate, a  $>1,400$ -bp fragment of the 16S rDNA gene was amplified using consensus primers corresponding to the U1 and U8 conserved sequences (11). The amplicon was cloned into the pCR2.1 sequencing vector (TOPO TA cloning kit; Invitrogen), and both strands were sequenced by automated dideoxy DNA methods. A sequence similarity check was performed by a BLASTN.2.2.3 search of the GenBank database (National Center for Biotechnology Information) (1). The isolate from the horse was most closely related to *H. ovis*, with an overall identity of 99.93% (1,422 of 1,423 nucleotides) to the published sequence (6), and was more distantly related to *H. kunzii*, with an overall identity of 95% (1,188 of 1,246 nucleotides), and to a *Peptostreptococcus* sp., with an overall identity of 92% (907 of 976 nucleotides). The phylogenetic position of this isolate was confirmed by performing 1,000 replicate bootstrapping analyses (SEQBOOT) and 100 replicate DNA parsimony analyses (DNAPARS) by using the PHYLIP package (8). Based on the 16S rDNA analysis, this organism was identified as *H. ovis*.

**Discussion.** *Helcococcus* has not been previously reported in horses. In this report, we identified *H. ovis* from a very large posttraumatic abscess in the lungs of a horse. Only one previous report described *H. ovis*; in that study the organism was found in two sheep (6). The findings in the present report indicate a wider distribution of the organism in veterinary species. Although the organism in this case differed slightly in its biochemical profile from the previously described *H. ovis* by being negative for the production of alkaline phosphatase, it was shown to be virtually identical to *H. ovis* based on the 16S rDNA gene sequence. The taxonomy of the catalase-negative, facultatively anaerobic gram-positive cocci is still poorly defined, and accurate identification of these bacteria is problematic. *H. kunzii* may be misidentified as *Aerococcus viridans* with the API 20S kit; however, *Aerococcus* can be distinguished from *Helcococcus* by the inability to grow anaerobically and by being nonlipophilic (7). *H. ovis* may be misidentified as *Abiotrophia* (*Granulicatella*) *adiacens* with the API 20S kit, though *Abiotrophia* can be distinguished from *Helcococcus* by poor growth on unsupplemented blood agar (5) and by the formation of cocci in chains. The increased use of 16S rDNA sequencing as a method of bacterial identification in the diagnostic laboratory should help to clarify the epidemiology and clinical significance of this group of organisms.

The presence of the isolate in large numbers and in a relatively pure culture from both the abscess and the transtracheal-wash samples indicated that it was most likely responsible for the abscess formation in this horse's lungs and acted as a

pathogen. In the previous report of the isolation of *H. ovis*, the organism was noted to be present in two sheep as mixed infections with other known ovine pathogens, and thus its pathogenic role was not clear (6). Since *Helcococcus* seems to be mostly an opportunistic pathogen in humans (3, 9), it is likely that this was also the case with this horse. We were unable to determine if previous cultures were performed, making it impossible to determine the origin or time of the initial *Helcococcus* infection in this horse's lung. Therefore, the source of the organism is unknown but was presumably skin flora that was introduced into the wound, either by the initial penetrating foreign object or by the placement and maintenance of multiple chest tubes, resulting in an opportunistic infection. The previous prolonged multiantibiotic therapy could also have selected for, and permitted the growth of, the *Helcococcus* sp. from a mixed infection. We were also unable to determine if the horse was systemically healthy at the time of infection or was already debilitated by the chronicity of the disease or by prolonged usage of antimicrobials. However, despite chronic and significant respiratory disease, this horse was in good body condition and was not known to have any immunocompromising conditions.

The typical antimicrobial sensitivity for *H. ovis* is not well known. The strains isolated from the sheep in 1999 were sensitive in vitro to vancomycin (6); however, this drug is not commonly used in equine medicine due to cost and judicious-use practices. The reported antimicrobial profile for *H. kunzii* includes susceptibility to vancomycin (3, 4, 9, 10), penicillin (3, 4, 10), flucloxacillin (10), and clindamycin and ampicillin (4). *H. kunzii* isolates showed intermediate susceptibility or resistance to ciprofloxacin in one study (3), and in another they showed resistance to erythromycin (4). If extrapolation of the antimicrobial susceptibility of *H. kunzii* to *H. ovis* is possible, resistance to ciprofloxacin and erythromycin could be disadvantageous in equine medicine, since these classes of antibiotics are commonly used for treating deep abscesses in horses. Although this horse was on penicillin for a prolonged period, the drug seemed to be unable to control the infection. This may have been due to the well-encapsulated nature of the abscess and poor penetration of the drug rather than to the resistance of the organism.

We were unable to test the isolate for susceptibility to trimethoprim-sulfadiazine, but this drug combination was selected in this case due to its broad spectrum, its good tissue penetration (2), and financial constraints. Although the in vivo efficacy of trimethoprim-sulfadiazine may be limited in the presence of large amounts of pus or necrotic tissue (2), in this case, the combination of surgical drainage and debridement of the abscess, flushes with 3% povidine solution, and parenteral administration of trimethoprim-sulfadiazine (30 mg/kg every 24 h) seemed sufficient to allow complete healing and resolution of the lesion.

Awareness of *Helcococcus* as a potential pathogen in equine veterinary medicine is important for prompting clinicians to request its culture, since it requires specific conditions not routinely used by most laboratories. Further, antimicrobial susceptibility testing is also necessary for a better understanding of this organism and to aid in selecting the appropriate drugs when treating *Helcococcus* infection. An earlier recognition of

the organism will provide the opportunity for a more rapid diagnosis, more specific treatment, and, consequently, a speedier improvement of the patient's condition.

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