Illustration of the Difficulty of Identifying *Streptococcus equi* Strains at the Subspecies Level through a Case of Endocarditis in an Immunocompetent Man

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We report a case of endocarditis caused by *Streptococcus equi* in an immunocompetent patient who was subsequently cured after appropriate antibiotic therapy and cardiac surgery. However, it was challenging to identify the strain to the subspecies level, which highlights the necessity of developing reliable molecular tools to discriminate between the subspecies.

CASE REPORT

In January 2012, a 63-year-old man presented to the emergency room with a 2-week history of low-grade fever but without other symptoms and reported self-treatment with paracetamol. Past medical history included pituitary adenoma, which was treated by surgery in 1989, and a mechanical aortic valve replacement in December 2008 as treatment for severe aortic insufficiency.

At admission, he had no fever (37.2°C), was under treatment with paracetamol, and had a pulse rate of 71/min. His blood pressure was 128/91 mm Hg, and oxygen saturation was 97% on air. An erythematous skin rash on his forehead extending to his right infraorbital area, which appeared a few hours before admission, was noted. No other skin lesions were apparent by physical examination. Cardiac auscultation was normal, and no signs of cardiac failure were found. Electrocardiogram revealed a second-degree atrioventricular block, while neurological, abdominal, and respiratory examinations were normal. The thoracic computed tomography scan was normal, transthoracic and transesophageal echocardiographies (TTE and TEE) did not show any vegetation or raphy scan was normal, transthoracic and transesophageal echocardiographies (TTE and TEE) did not show any vegetation or signs of endocarditis, and the mean transaortic valve gradient was close to 35 mm Hg without pathological regurgitation.

Initial laboratory investigations revealed a leukocyte count of 10,100/mm³ (75% neutrophils) (8,000 to 10,000/mm³), the C-reactive protein concentration was 30 mg/liter (normal, and Gram-positive coccus chain formation suggestive of *eux*). Aerobic and anaerobic bottles (eight out of nine sets) were performed daily, and cultures were incubated in the BacT/Alert automated system (Organon Teknika, bioMérieux). Gram staining demonstrated thin, short to medium-length, Gram-positive, non-spore-forming cocci suggestive of *Streptococcus*. The strain was identified as Lancefield group C, and rapid ID 32 Strep and API 20 Strep (bio-Mérieux) results were *Streptococcus equi* subsp. *zooepidemicus* (probability, 99.7%). However, the following phenotypical characteristics led to an identification as *Streptococcus equi* subsp. *ruminatorum*: the CAMP reaction was positive, and the strain hydrolyzed hippurate, fermented ribose, and did not acidify methyl β-D-glucopyranoside (1, 2, 3).

To confirm the identification to the subspecies level, the strain was further analyzed by molecular testing. All except one gene led to an identification as *Streptococcus equi* subsp. *ruminatorum*. Bacterial identification of the strain was performed by 16S rRNA sequencing as previously described using the fD1 and rp2 primer pair (4). We obtained a 1,414-bp sequence, which was compared to sequences in the GenBank database using the BLAST algorithm; the best score corresponded to *S. equi* subsp. *ruminatorum* (GenBank accession number EF406035; 1,405/1,405 bp, 100% similarity), and the second-best score was to *S. equi* subsp. *zooepidemicus* (GenBank accession number EF406023; 1,404/1,405 bp, 99.9% similarity). Considering the large number of sequences for these two subspecies available in GenBank, we decided to compare our sequence to those in a curated database containing only sequences of type strains, since noncurated databases can contain misidentified sequences due to erroneous identifications, as previously reported (5). Using the type strain database available on the BIBI website (6), we found 100% similarity to *S. equi* subsp. *ruminatorum* (GenBank accession number EF406035; 1,405/1,405 bp) but 98.6% similarity to the next-closest subspecies, *S. equi* subsp. *zooepidemicus* (GenBank accession number FM204884; 1,390/1,410 bp). To narrow the results, three additional targets were amplified: a 370-bp sodA fragment by using the primers sodA-up and sodA-dn (7), a 645-bp rpoB fragment by using the primers...
S. equi is comprised of three subspecies, S. equi subsp. equi, S. equi subsp. zooepidemicus, and S. equi subsp. ruminatorum. Identification of S. equi subsp. equi is easy to perform, whereas identification of S. equi subsp. ruminatorum and S. equi subsp. zooepidemicus is more difficult, as illustrated in this case. Since S. equi subsp. ruminatorum is closely related to S. equi subsp. zooepidemicus, reliable discrimination between S. equi subsp. zooepidemicus and S. equi subsp. ruminatorum on the basis of biochemical characters and molecular biology is controversial (1, 2, 3).

Therefore, some of the cases attributed to S. equi subsp. zooepidemicus may be due to S. equi subsp. ruminatorum, making review of the literature difficult.

S. equi subsp. equi and subsp. zooepidemicus are common pathogens in veterinary medicine. S. equi subsp. equi is the causative agent of equine strangles (10), and S. equi subsp. zooepidemicus is a commensal of horses' upper airways and can also cause wound, respiratory, and uterine infections in those animals (10). Rare cases of human transmission, leading to pneumonia (11), bacteremia (11, 12, 13), septic arthritis (11, 13), meningitis (12), toxic shock-like syndrome (13), spondylodiskitis (14), infection of vascular grafts or aneurysm (15), and endocarditis (11, 16), have been reported. Cases previously described resulted from inhalation, inoculation (12), or ingestion of inadequately pasteurized products, which led to outbreaks (11). S. equi subsp. equi and subsp. zooepidemicus share approximately 80% genome sequence identity with Streptococcus pyogenes and have many virulence factors in common with this organism (12).

S. equi subsp. ruminatorum was identified in 2004 in cases of mastitis in small ruminants (1) and was subsequently isolated from zebras (2), spotted hyenas (2,17), and African wild dogs (18) in Tanzania. There are to date only a few publications on which we can rely for S. equi subsp. ruminatorum. Indeed, a literature review revealed only two previously reported cases of S. equi subsp. ruminatorum human infections (19, 20). The demographics, probable routes of acquisition, molecular methods used to identify ruminatorum subspecies, treatments, and outcomes of the described cases, including those of our patient, are summarized in Table 1. It can be highlighted that the strain in each of those previous cases was identified as S. equi subsp. ruminatorum by a single molecular tool, unlike with our strain. The previous cases occurred in immunocompromised men [with HIV or IgG(κ) monoclonal gammopathy]; one of these men died within 2 days from an uncontrolled disseminated infection and the other recovered after endocarditis complicated by spondylodiskitis was treated (19, 20). Beta-lactam and aminoglycoside association was used in 2 out of the 3 cases reported in Table 1. Beta-lactam antibiotic, particularly penicillin, are considered agents of choice for group C streptococcal infections (9). In severe infections, such as endocarditis, the addition of aminoglycosides may result in more favorable outcomes (9). Neither of the two patients who received beta-lactam antibiotics died. The mortality rate for patients with infective endocarditis is about 16% (9) (14% in cases of infective Streptococcus milleri endocarditis and 25% in cases of infective beta-hemolytic Streptococcus endocarditis [21]). S. equi subsp. ruminatorum may cause serious infections in both immunodeficient and immunocompetent patients (19, 20). Infections due to this microorganism are probably zoonoses, despite the fact that the rate of human-to-human transmission remains unknown. Of those three cases, two, including the patient in this study, had contact with horses. Our patient reported regular contact with horses, as he accompanied his daughter to horseback-riding out-
Normal count 39 Blood gene sequencing Amoxicillin (200 mg/kg/J) / H11001

Bacteremia, meningitis, 9,600 (133 CD4 38.9 Blood, CSF, 16S rRNA gene sequencing Cefotaxime (12 g/J) vancomycin

Recovered

Mitral valve endocarditis Occasional contact with horses; inoculation (skin lesion) complicated by spondylodiskitis Prophylactic-antiviral value of antibiotics

Phenotypic classification remains controversial (1, 2, 3), and the use of molecular targets to discriminate between S. equi subsp. zooepidemicus and subsp. ruminatorum, in particular, the inability of 16S rRNA sequencing to discriminate between the subspecies (2, 3), is not consensual. A multilocus sequence typing method has been developed for S. equi subsp. zooepidemicus (22), but subsp. ruminatorum sequences are lacking. As for virulence genes, there are szp (encoding M-like protein) sequences for this subspecies in the GenBank database. Based on our isolate, phenotypical data and molecular data, with the exception one of the molecular targets (i.e., szp), are mostly suggestive of S. equi subsp. ruminatorum. This highlights the difficulty in identifying S. equi to the subspecies level and the necessity of (i) developing reliable molecular tools to discriminate between subspecies and (ii) curating the available released sequences that can lead to erroneous identifications.

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REFERENCES


### TABLE 1

<table>
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<tr>
<th>Case</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Past medical history</th>
<th>HIV infection</th>
<th>Route of acquisition</th>
<th>Antibiotics</th>
<th>Molecular testing</th>
<th>Outcome</th>
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<tr>
<td>15</td>
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<td>No</td>
<td>Amoxicillin</td>
<td>16S rRNA gene sequencing</td>
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<td>16S rRNA gene sequencing</td>
<td>Recovered</td>
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<td>No</td>
<td>No</td>
<td>Cefotaxime</td>
<td>16S rRNA gene sequencing</td>
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