Case Report

**Arcanobacterium pyogenes** Spondylodiscitis in a Veterinary Surgeon: a Plea for Cooperation between Medical and Veterinary Microbiologists in Identification of Causal Agents of Zoonotic Infections

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**Introduction**

Zoonotic diseases are an ever-present concern in veterinary practice and may be a problem in human clinical microbiology laboratories because of their unfamiliarity as causal agents of disease. A case of spondylodiscitis caused by *Arcanobacterium pyogenes* in a veterinary surgeon, the first reported human case, prompted us to collect rare and difficult-to-identify causes of zoonoses encountered in our hospital during the past years. We present four cases with rather unusual zoonotic agents: *Arcanobacterium pyogenes*, *Streptococcus suis*, *Erysipelothrix rhusiopathiae*, and *Actinobacillus suis*, well-known pathogens to the veterinary microbiologist but subject to possible misidentification in the human microbiology laboratory.

**Case Reports**

**Case 1**

A 56-year-old male veterinarian was referred to our hospital with a 6-week history of low back pain. He had a history of a cerebrovascular accident, implantation of two knee prostheses following osteoarthritis, and hyper-
cholesterolemia. His medications were 160 mg of acetylsalicylic acid per day, 75 mg of clodipogrel per day, and 40 mg of pravastatin per day. The patient had extensive contact with cattle and swine and did not wear gloves. He regularly suffered from excoriations on both hands. Since treatment with oral sodium diclofenac did not relieve his back pain, he consulted an orthopedic surgeon and was treated with a series of 10 epidural infiltrations of methylprednisone, as well as oral sodium diclofenac. Blood analysis ordered by his general practitioner revealed anemia with a hemoglobin level of 10.5 g/dl (normal range; 14 to 18 g/dl), a white blood cell count of 8,000/µl (normal range; 4,000 to 9,000/µl) with 78.4% polymorphonuclear cells, an elevated erythrocyte sedimentation rate (ESR), and an increased C-reactive protein (CRP) level of 145 mg/l (normal range; 0.02 to 7 mg/l). Screening for autoimmune antibodies was negative. The patient was HLA B27 negative. Conventional X rays showed spondylodiscitis of the L3-L4 interspace. Nuclear magnetic resonance imaging showed significant destruction of lumbar vertebrae L3 and L4 with some minor abscess formation in the intervertebral disk, in addition to limited epidural inflation. Transthoracic echocardiography excluded endocarditis. Transcutaneous puncture of the lumbar joint L3-L4 yielded a bloody fluid with streptococcus-like gram-positive bacteria seen on Gram-stained smear. Culture on horse blood agar grew beta-hemolytic colonies reacting with a Lancefield group G antigen reagent (Pro-Lab Diagnostics, Neston, Wirral, United Kingdom), which erroneously led to further processing of the isolate as a streptococcus. Molecular identification at the Laboratory of Veterinary Bacteriology and Mycology (Faculty of Veterinary Medicine, Ghent University), by using 16S rRNA intergenic length polymorphism analysis (tDNA-PCR) combined with capillary electrophoresis (1) characterized the organism as A. pyogenes. The identification was confirmed in house using 16S rRNA gene sequencing. In a former case of a farmer suffering from S. suis endocarditis, the patient was successfully treated with 6 × 4 MIU of penicillin G/day i.v. for 4 weeks and gentamicin (dosage adjusted to the blood levels) for 10 days, in combination with aortic valve replacement. In our most recent case, we started treating the patient with ampicillin (3-11). However, because the condition of our patient did not improve and CRP levels increased, we suspected that ampicillin did not penetrate into the hip joint. The patient was switched to 900 mg rifampin/day per os (p.o.), in combination with 1,200 mg linezolid/day p.o. He recovered quickly and left the hospital 2 weeks later. He continued the same therapy at home.

Case 2

A 64-year-old farmer was admitted to our hospital. The patient was injured handling the feed trough of his pigs. He limped and had fever. He had a history of type 2 diabetes mellitus, right heart failure, a myocardial infarction with an unstable coronary syndrome, and an implantation and reimplantation of a left hip prosthesis. His medications were 5 mg of amldipine per day, 160 mg of acetylsalicylic acid per day, 50 mg of losartan potassium per day, 20 mg of simvastatin per day, 5 mg of bisoprolol hemifumarate per day, 8 units of human biosynthetic insulin 20/80 in the morning, and 20 units of human biosynthetic insulin 40/60 in the evening. Blood analysis revealed a normal hemoglobin level of 13.9 g/dl (normal range, 13.3 to 16.7 g/dl), an elevated white blood cell count of 13,900/µl, an elevated ESR, and an increased CRP level of 190 mg/l. Conventional X rays showed a radiolucent area around the implant, suggesting aseptic loosening of the prosthesis. Bone scintigraphy showed a diffuse hypercaptopation, also compatible with aseptic loosening of the hip prosthesis. Endocarditis was excluded by transesophageal echocardiography. Blood cultures yielded gram-positive cocci, which grew on sheep blood agar as alpha-hemolytic colonies reacting weakly with a Lancefield group D antigen reagent (Pro-Lab Diagnostics). The API 20 Strep system (bioMérieux SA, Marcy l’Etoile, France) confirmed the identification of A. pyogenes (API Coryne profile 4712760, 99.9% probability). The patient was treated with penicillin G, 6 × 3 MIU/day i.v., and clindamycin, 2 × 600 mg/day i.v., for 32 days. His lumbar condition improved quickly, but he developed a painful effusion in his knees without microbiological evidence of infection. Nevertheless, he was treated with fluocoxacinil and clindamycin for 16 days. He was discharged after 7 weeks of hospitalization.

Case 3

This case has been reported previously (12). A 45-year-old mentally retarded male was admitted to our hospital with dyspnea. On admission, he was afebrile. He suffered from several itching excoriations on the chest skin. His medical history revealed alcohol abuse and progressively deteriorating renal function due to a congenitally absent left kidney and a multicystic right kidney. The patient had no pets and he denied any animal exposure. He was working as a gravedigger. Laboratory investigations indicated end-stage renal disease and showed a white blood cell count of 8,170/µl, hemoglobin 7.2 g/dl (normal range; 12 to 18 g/dl), and a CRP level of 53.1 mg/l. Hemodialysis was started on the second day of hospitalization. Fever to 38°C developed on day 8 of hospitalization; blood cultures were drawn, a central venous catheter was placed, and 1 g of amoxicillin-clavulanate (AMC) q 6 h was started. His temperature decreased. The blood cultures yielded gram-positive rods after 20 h of incubation. The isolate was identified as E. rhusiopathiae based on the basis of the following characteristics: catalase and oxidase negative, alpha-hemolytic colonies on blood agar, H2S production on a triple-sugar iron agar slant, vancomycin resistance, and penicillin susceptibility with a MIC of 0.064 mg/l (using the CLSI [formerly NCCLS] breakpoints for Enterococcus spp.). Identification was confirmed by API Coryne (API Coryne V 1.1 profile 4120100; 99.9% probability) and at the...
Laboratory of Microbiology, Catholic University of Leuven (J. Verhaegen). Repeated transesophageal echocardiography showed a large vegetation on the anterior mitral leaflet, confirming endocarditis. The amoxicillin-clavulanate was discontinued and replaced by 1 g of ampicillin q 8 h for 3 weeks. The total duration of antimicrobial treatment was 28 days; cultures became negative after 2 weeks of treatment. The patient was discharged, and mitral valve surgery was performed 4 weeks later. The patient’s postoperative recovery was uneventful. At the 2-year follow-up visit, the patient was still receiving hemodialysis for end-stage renal failure, but there was no recurrence of the infection.

Case 4
A 68-year-old male patient was referred to our hospital by his general practitioner. The patient, who had no previous medical problems, was bitten on his right knee by his Vietnamese potbelly pig. He developed a septic arthritis. Blood analysis revealed a white blood cell count of 13,710/µl with 82.5% polymorphonuclear cells, an increased ESR, and a CRP level of 74 mg/l. Knee aspiration yielded a purulent effusion and was followed by rinsing of the joint. Intravenous AMC (6 × 1 g daily) was started. A Gram-stained smear of the effusion showed numerous polymorphonuclear cells, but no bacteria were present. The fluid was cultured on selective and non-selective media, but only a few adherent beta-hemolytic colonies were observed on horse blood agar. Subculture onto Mueller-Hinton agar grew the same gray, sticky colonies. In culture, the organisms were nonmotile, gram-negative, slender cocccobacilli. Further testing showed the bacteria were facultatively anaerobic, catalase negative, oxidase positive, urease positive, indole negative, methyl red positive, Voges-Proskauer negative, ornithine decarboxylase negative, and reduced nitrate to nitrite. Esculin hydrolysis was positive, gelatin hydrolysis negative, and o-nitrophenyl-β-D-glucopyranoside (test for beta-galactosidase) positive. Growth on Kligler iron agar gave an acid/acid appearance similar to that obtained with Pasteurella spp. Inoculation of API 20 E (bioMérieux, Marcy l’Etoile, France) produced acidification of amygdaline, glucose, sucrose, arabinose, and melibiose but no reaction with mannitol, inositol, sorbitol, and rhamnose. Presumed identification of Actinobacillus suis was confirmed by the Global Infectious Diseases & Epidemiology Network (GIDEN) software (100%) and by J. Verhaegen at the Laboratory of Microbiology of Catholic University of Leuven. The patient was treated successfully and left the hospital one week later with 500 mg p.o. AMC t.i.d.

Microbiology
Arcanobacterium pyogenes
A. pyogenes, formerly referred to as Actinomyces pyogenes, is one of five species in the genus Arcanobacterium, of which Arcanobacterium haemolyticum is the best known in human medicine. It may be recovered, although rarely, from throat samples and is associated with pharyngitis (13-17). A. pyogenes is a well-established cause of infections in farm animals. It can also be isolated from healthy animals but is not a part of the normal human flora (14,15,17-19). Culture morphology is very similar to that of beta-hemolytic streptococci, and misidentification is possible. Agglutination with streptococcal group G antiserum may further confound the correct identification. Key features are the presence of small gram-positive rods with pointed ends on Gram stain, pinpoint colonies, and weak hemolysis after 24 h on sheep blood agar in a CO₂-enriched environment. The colonies grow to a 1-mm diameter large form with a sharp zone of beta-hemolysis after 48 h of incubation. There is no distinctive smell or pigment. The catalase reaction is negative, there is no motility, and the CAMP test is positive, whereas A. haemolyticum shows inhibition of the Staphylococcus aureus-induced hemolysis (13-16,18,20). The beta-glucuronidase reaction is positive in 11 to 89% of the strains (21). Gelatin is hydrolyzed, and xylose is fermented; these three tests are included in the API Coryne strip which makes it a useful tool for correct identification (13,14,16,18,20,21).

Streptococcus suis
S. suis is the name assigned to bacteria formerly called Lancefield groups R, S, RS, and T streptococci. Kilpper-Balz and Schleifer (8,22) assigned these strains (R, S, RS, and T) and several other serotypes with the same pheno-
primarily been seen as a veterinary pathogen. Erysipeloid is a well-known disease in pigs, but other animals may also be affected (16,30-34). The genus Erysipelothrix contains two species: E. rhusiopathiae and Erysipelothrix tonsillarum. Sucre assimilation may distinguish between the two species: E. tonsillarum is positive, whereas E. rhusiopathiae is sucore negative. Only E. rhusiopathiae has been recovered in humans (16,30). Historically, E. rhusiopathiae in humans has been associated with a cutaneous inflammatory disease often affecting the hands and fingers. It follows occupational exposure to animals and their products, mostly swine and fish (32,35-37). Although the organism cannot persist in the environment indefinitely, soil that has been contaminated with E. rhusiopathiae can yield positive cultures for many weeks. Viable organisms have been recovered from a buried carcass after 9 months of interment (30). Grave digging may be a risk factor not reported until now. Poor personal hygiene, end-stage renal disease, and alcoholism may have contributed to the development of the infection in our patient. Previous alcohol abuse was mentioned in 33% of patients developing E. rhusiopathiae endocarditis, which makes it the most commonly reported underlying condition in these patients (33). More recently, it has been linked to various other clinical syndromes, such as polyarthralgia with renal failure, septic arthritis, peritonitis, and necrotizing fasciitis (30). Invasive infections with this organism are unusual. Invasive endocarditis with the potential for significant morbidity and mortality has been reported (30,32,33, 35,37-41), often necessitating valve replacement. Since Gram stain, colony morphology, and negative catalase results might suggest common laboratory isolates, such as Lactobacillus spp., Actinomyces spp., Streptococcus spp., or even Enterococcus spp., definitive identification of E. rhusiopathiae may be a challenge for the clinical microbiologist (31,38). A key test is diffuse, dust-like H₂S precipitation in triple-sugar iron agar.

**Actinobacillus suis**

The genus Actinobacillus belongs to the family Pasteurellaceae, often isolated in the human medical microbiology laboratory from samples of animal-associated injuries (16,42,43). Most Actinobacillus spp. belong to the commensal oropharyngeal flora of animals (cattle, sheep, horses, and rabbits) and are rarely isolated from asymptomatic humans (42-44). Only Actinobacillus actinomycetemcomitans is better known in human microbiology, but it does not belong to the genus Actinobacillus sensu strictu (16). Actinobacillus spp. are facultatively anaerobic, nonmotile, fastidious gram-negative rods, appearing as coccoid to short rods if cultured on routine solid media and as longer rods when cultured in serum broth and on media containing sugars. They show a tendency toward bipolar staining. Arrangement is single, in pairs, and in short chains. Differentiation from Pasteurella spp. depends on a wide range of tests. In the reported case, only the negative catalase reaction gave us the key to correct identification, although the literature mentions that this test may be variable for A. suis. A. suis can cause a variety of diseases in horses and pigs. Human infections are mostly due to horse- and pig-associated injuries (16,42,44).

**Conclusion**

Zoonotic diseases are an ever-present concern in veterinary practice and may be overlooked or misidentified in human microbiology laboratories. Awareness and retrospective data are of great importance. Following the diagnosis of spondylodiscitis due to A. pyogenes in a veterinary surgeon, we reported three other infections due to various zoonotic agents: S. suis, E. rhusiopathiae, and A. suis. In each case, knowledge of a link with animals or organic material could have helped the medical microbiology laboratory identify the causative agent correctly and rapidly. Once again, communication between clinicians and the laboratory proved to be crucial. Treatment is often a combination of surgical management, such as debridement and good wound care, and administration of appropriate antimicrobial agents. Usually, broad-spectrum antibiotics are started (45), but after correct identification, therapy can be streamlined according to the antimicrobial susceptibility report.

**References**


