Streptococcus equi subspecies *zooepidemicus* Infections in Humans by Zoonotic Transmission from Horses

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

Streptococcus equi subspecies *zooepidemicus,* or *S. zooepidemicus*, is a zoonotic pathogen for people in contact with horses. In horses, *S. zooepidemicus* is an opportunistic pathogen, but human infections associated with *S. zooepidemicus* are often severe.

Within six months in 2011, three unrelated cases of severe, disseminated *S. zooepidemicus* infections occurred in men working with horses in eastern Finland. We describe the clinical presentation of three cases and compared the *S. zooepidemicus* isolates from the human cases with *S. zooepidemicus* isolates from horses to obtain insight into the epidemiology of *S. zooepidemicus*. The isolates were analyzed using pulsed-field gel electrophoresis, multilocus sequence typing and sequencing of the *szP* gene. The molecular typing methods showed that human and equine isolates were identical or closely related. These results emphasize that *S. zooepidemicus* transmitted from horses can lead to severe infections in humans. As leisure and professional equine sports continue to grow, this infection should be recognized an as emerging zoonosis.

S. zooepidemicus has seldom been isolated from humans. Surprisingly, the majority of published data on humans goes back to the latter part of the 1980s. Occasional human infection was reported as a result of the consumption of homemade cheese or unpasteurized milk from cows with mastitis. In humans, *S. zooepidemicus* may cause glomerulonephritis and rheumatic fever, which are known sequelae of *Streptococcus pyogenes* infections. Meningitis and purulent arthritis have also been reported.

S. zooepidemicus displays a wide genetic variation between different isolates. The sequence of the SzP protein gene, or *szP,* has been shown to vary greatly between different strains of *S. zooepidemicus* and the variable regions of *szP* can be used to genetically differentiate strains within the subspecies. Pulsed-field gel electrophoresis, or PFGE, is a DNA-based typing technique that is highly discriminatory and has been used in epidemiological investigations of *S. zooepidemicus* outbreaks.

Within a six-month period, through our routine practice, we found three cases of severe disseminated disease in humans caused by *S. zooepidemicus.* The purpose of this study was to characterize the clinical presentation of the disease caused by *S. zooepidemicus*, microbiologically characterize the isolated strains, and identify clonality of human isolates for comparison to equine isolates from contact horse stables or other horse farms of the surrounding area.

Patient 1

A 57-year-old male farmer and horse breeder from eastern Finland was admitted unconscious and febrile to the emergency room in February of 2011. He was intubated, a lumbar puncture was performed, and cerebrospinal fluid, or CSF, was collected before his transfer to the intensive care unit. His medical records showed an insufficiency of his aortic valve and that he had been

catheterized three months earlier as a preoperative measure. His condition was septic with clinical symptoms of meningismus and pulmonary congestion. Staining of the CSF revealed gram-positive cocci in chains with a considerable number of polymorphonuclear cells on the microscopic examination. The next day, *S. zooepidemicus* was cultured from the CSF and four of the four blood culture bottles, leading to a primary diagnosis of meningitis and sepsis. Intravenous high dose penicillin treatment, for five weeks, was started in combination with gentamicin, for the first 10 days. Two and a half days after admission, the patient regained consciousness. Intravascular coagulopathy developed and 20 days later, progressive endocarditis. The bicuspid native aortic valve was resected the same day, and several bacterial patches were observed. His perioperative blood cultures remained negative. Neurologic sequelae did not develop but his recovery and rehabilitation required several weeks.

Patient 2

A 62-year-old-male truck-driver and horse trainer from eastern Finland returned home from work febrile and confused in May of 2011. The next day, medical examination revealed pain and swelling of his right knee and right shoulder, hyperglycemia, and untreated non-insulin dependent diabetes mellitus. The synovial fluid aspirated from his knee was turbid with an elevated leukocyte count and a high percentage of polymorphonuclear cells. Samples for blood and synovial fluid cultures were obtained and anti-microbial therapy with intravenous cefuroxime was started. *S. zooepidemicus* was cultured from his right knee. On the third day, arthroscopic synovectomy and irrigation of the right knee was performed and the procedure was repeated. Cefuroxime was changed to intravenous vancomycin without therapeutic response. Next, a combination of penicillin G with clindamycin was administered. He had no signs of endocarditis, but scintigraphy revealed an uptake in the patient's right shoulder and lower jaw region. Arthroscopic debridement and irrigation of the right shoulder was performed and purulent synovial fluid was collected for culture. Antimicrobial therapy continued with intravenous cefuroxime and clindamycin for two weeks; thereafter oral cephalexin and clindamycin for one week. The patient's clinical condition gradually improved and finally he was able to walk with crutches. He was discharged from the primary care hospital six weeks after the onset of illness.

Patient 3

A 49-year-old male horse trainer from eastern Finland was admitted to the hospital in August of 2011 because of severe, prolonged low-back pain. A horse had kicked his forehead two to three weeks earlier. The accident did not require medical attention at that time, however. The low-back pain had increased gradually. He had medicated himself with ibuprofen 400 mg up to 20 tablets per day without relief, except when lying supine. He did not record his temperature but was sweating after taking ibuprofen and sought medical attention when walking became difficult. His condition was treated as muscle pain. After a week he returned to the medical center because of excruciating pain in his back. There were no abnormal radiological findings. On clinical examination, he was well and non-febrile and had no hemodynamic abnormalities. The clinical findings were unremarkable except for the pain in his lower back on percussion and a pulsating abdominal mass. Laboratory tests showed leukocytosis, elevated erythrocyte sedimentation rate, and an elevated CRP. Computed tomography revealed a psoas-abscess linked to an infected aortic aneurysm. The aneurysm was resected, replaced by a Y-prosthesis, and the psoas-abscess was drained. The patient's condition was treated with piperacillin-tazobactam, later replaced with intravenous

penicillin. Transesophageal echocardiography showed no signs of endocarditis. The patient recovered without sequelae.

Collection and Microbiological Characterization of Equine Isolates

None of the horses from the stables associated with the first two human cases (patients 1 and 2) showed any sign of respiratory illness. The horses from the third stable (owned by patient 3) were not examined; however, the owner did not recall any clinical signs of respiratory or other disease in his horses. Nasal swab specimens were collected from seven horses owned by patient 1 (stable A), and 4 horses owned by patient 2 (stable H). The swabs were streaked onto bovine blood agar plates and incubated for 24 hours. *B*-hemolytic colonies were studied with conventional methods and biochemical characterization was performed. *S. zooepidemicus* was isolated from five horses in stable A, but not from any horse in stable H. Six other *S. zooepidemicus* isolates from horses unrelated to the described human cases were included in the genetic comparison.

Microbiological Identification and Antibiotic Susceptibility of Human Isolates

The colonies of *S. zooepidemicus* on blood agar were large, mucoid, and had a wide zone of β-hemolysis. All isolates were sensitive to erythromycin, clindamycin, penicillin, vancomycin, and cephalexin.

Discussion

We report three unrelated cases of *S. zooepidemicus* infection in patients from eastern Finland who had close and continuous contact with horses. It is noteworthy that the disease in all three patients was invasive and severe, requiring prolonged treatment and rehabilitation. Sepsis occurred in two cases (patients 1 and 2), meningitis and endocarditis in one (patient 1), purulent arthritis in one (patient 2), and a psoas abscess in connection with an aortic wall infection in one case (patient 3). In patient 3, transient bacteremia might have occurred earlier.

MLST, PFGE, and sequencing of the SzP protein gene demonstrated identical profiles in a human isolate (Hum1) with an equine isolate, which strongly supports the zoonotic nature of this disease. Notably, the strain colonized the horse's nostrils and acted as an innocent commensal, whereas in humans this strain appeared highly virulent and caused severe illness. In the second case (patient 2) we were unable to isolate the same strain from his horses. This failure may have been due to a transient *S. zooepidemicus* carriage in the nasopharynx, lymphoid tissues, or respiratory tract of the horse. Patient 2 might have been in contact with other horses as well. The strains from patient 1 and patient 2 were identical according to both *szP* sequencing and MLST, which supports the close relationship between the Hum1 and Hum2 isolates, and although the two isolates differed on PFGE analysis, the data strongly suggest that the infection of patient 2 was also transmitted zoonotically.

All strains of *S. zooepidemicus* displayed mucoid colonies on the agar plates, indicating expression of a hyaluronic acid capsule, a well-known virulence factor in other pathogenic streptococci, such as *Streptococcus equi* in horses and *Streptococcus pyogenes* in humans. However, the expression of the mucoid capsule was variable: Hum1 strain produced very large and highly mucous colonies, whereas those from Hum3 were heterogeneous in colony size and less mucoid. Whether there is a correlation between the production of mucinous substance and severity of the disease remains to be determined. Additional virulence factors, such as the presence of S antigens, would be most intriguing to investigate. In *Strep pyogenes,* variation in the M-protein is attributed to variable

virulence. For example, the M1 strains are the most pathogenic. The sequence variants of the SzP protein gene in *S. zooepidemicus* could not be correlated with clinical features in horses in a study by Walker and Runyan. However, determining such a correlation might be possible for the human isolates.

Recently, an outbreak of invasive *S. zooepidemicus* infection has been reported from Finland. Altogether, seven patients were identified: six with septicimia and one with purulent arthritis. All had consumed goat cheese produced from unpasteurized milk in a small-scale dairy. In Finland (a population 5.2 million), all invasive streptococcal infections must be reported to the National Infectious Disease Register. Only three cases of invasive *S. zooepidemicus* infections were reported to the register between 1992 and 2002, and approximately 10 cases of invasive group C streptococcal infections occurred annually. In other words, even invasive isolates were often typed only to the Lancefield group level.

The novelty of our investigation is that an identical S. *zooepidemicus* strain was isolated from patient 1 and from a healthy horse in his stable, suggesting zoonotic transmission. Furthermore, patient 2 was infected with a S. *zooepidemicus* strain clonally related to that of patient 1, as judged by two independent typing methods described above, although patients 1 and 2 lived 140 kilometers apart without a verified contact with each other. Notably, this strain was highly virulent in humans but did not cause any clinical infection in the horse. In contrast, the isolate from patient 3 had the same MLST type as the strain previously isolated from several horses in an outbreak of respiratory disease. Our work yielded three new sequences of the *szP* gene.

Conclusion

Leisure and professional equine sports activities are growing in many countries. *S. zooepidemicus* infection transmitted from horses may cause severe illness in humans and should be considered as an emerging zoonosis.

Bacteriological identification of *S. zooepidemicus* is cheap and feasible with simple fermentation methods. Therefore, typing to the species level is strongly recommended for all clinical laboratories whenever group C streptococci are recovered from severely infected persons. Early identification of *S. zooepidemicus* will facilitate appropriate medical intervention and timely epidemiologic surveillance, and finally, prevent the spread of a potentially life-threatening pathogen.

I'm Dr. Mike Miller, for *Emerging Infectious Diseases*, and I've been reading an abridged version of *Streptococcus equi* subspecies *zooepidemicus* Infections in Humans by Zoonotic Transmission from Horses. You can read the entire article online in the July 2013 issue of *Emerging Infectious Diseases* at cdc.gov/eid.

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