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A Novel α -Hemolytic *Streptococcus* Species (*Streptococcus azizii* sp. nov.) Associated with Meningoencephalitis in Naïve Weanling C57BL/6 Mice

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Abstract

Streptococcus species adversely affect both immunocompetent and immunocompromised mice.^{2,16,30,31} As commensal bacteria, these gram-positive cocci inhabit the nose, mouth, intestine, genital tract, and skin. In general, pathogenic streptococci belong to Lancefield groups A, B, C, and G and are associated with suppurative lesions in the kidneys, heart, spleen, liver, uterus, thorax, lymph nodes, and lungs of affected mice.² C57BL/6 mice serve as a well-characterized model for *S. pneumoniae*, the cause of pneumococcal disease in humans.²⁵ Although C57BL/6 mice are highly susceptible to experimental infection with α -hemolytic *Streptococcus pneumoniae*, natural infections remain undocumented.² In humans, 10 of the most common subtypes of *S. pneumoniae* account for 62% of invasive bacterial disease worldwide and can cause clinical syndromes ranging from meningitis to pneumonia. Mouse models have been instrumental in developing pneumococcal vaccines for at-risk populations.²⁵

Many of the *Streptococcus* species that cause dental caries and that can lead to bacteremia are α -hemolytic, such as *S. mutans* and *S. sanguinis*.¹² In addition, *S. suis* causes clinical signs in swine similar to those of *S. pneumoniae* in humans and poses a significant zoonotic risk.^{34,35} Naturally occurring disease caused by other α -hemolytic *Streptococcus* species is extremely rare in mice.³⁰ When described, these infections occurred in immunodeficient mice, and, to our knowledge, there are no reports of α -hemolytic *Streptococcus* species causing clinical disease in immunocompetent mice.

In this report, we describe meningoencephalitis associated with a novel α -hemolytic *Streptococcus* species in a colony of C57BL/6NCrl mice. The bacterium appeared to be enzootic in at least some of the vendor's barrier breeding colonies. The pathogenesis of the disease associated with this bacterium remains to be fully elucidated.

Materials and Methods

Case History.

The index cases included a 30-d-old male C57BL/6NCrl (B6) mouse, which was runted, in poor body condition, and exhibited neurologic signs including an abnormal hopping gait, tremors, and hyperactivity. Four additional male weanling mice of the same approximate age but from different dams, from the same colony, and housed in cages on the same side of a double-sided

rack were found dead on the same day as the index case was discovered. A CBC analysis and gross and histopathologic evaluation were performed on the live index case. In addition, a section of formalin-fixed paraffin-embedded kidney from this animal was submitted for the detection of adventitious infectious agents by PCR assay (PCR Rodent Infectious Agent Plus Panel, Charles River Research Animal Diagnostic Services, Wilmington, MA).

After the index cases presented, the laboratory reported markedly increased neonatal and weanling death, accompanied by a consistent clinical syndrome of runting, hunched posture, lethargy, abnormal gait, and seizure-like activity in mice of the same signalment. During additional questioning, laboratory personnel retrospectively reported mortality as high as 50% in B6 weanlings (23 to 33 d of age) born to timed-pregnant dams (shipped overnight at embryonic day 17 to 18) from a single vendor over the prior 3 to 4 mo. During the next year, additional cases (12 male and 2 female mice) with the same signalment presented with similar clinical signs. In addition, 2 adult timed-pregnant mice and one 86-d-old male mouse presented with similar clinical signs. Representative animals were submitted for complete necropsy (weanlings, $n = 11$; dams, $n = 4$) or partial necropsy ($n = 7$) and aerobic and anaerobic bacterial culture ($n = 22$). In addition, selected affected weanlings ($n = 4$) and dams whose offspring were affected ($n = 4$) were screened by immunoassay (Multiplexed Fluorometric Immunoassay, Laboratory of Comparative Pathology, Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, New York, NY) for antibodies to the adventitious infectious agents evaluated during colony health monitoring.

Animals.

Timed-pregnant (embryonic day 17 to 18 on arrival) female C57BL/6NCrl mice (Charles River Laboratories, Wilmington, MA) were procured for use in a single laboratory's behavioral studies. No animals were purchased to conduct the investigation of the origins of the novel *Streptococcus* species described in this manuscript. All experiments were approved by Weill Cornell Medical College's IACUC. The animals were obtained from 5 barriers at 4 geographic sites. On arrival, the timed-pregnant dams were housed individually until parturition, after which they were housed with their litter until weaning. At weaning, litters were sexed, and male mice were retained and grouped into age-specific cohorts (5 mice per cage) for experimental purposes. Female weanlings were not retained in the colony, except during the investigation of the outbreak.

Mice were maintained in an AAALAC-accredited facility on a 12:12-h light cycle within individually ventilated, polysulfone, shoebox cages (no. 9, Thoren Caging Systems, Hazelton, PA) on autoclaved aspen chip bedding (Nepco, Warrensburg, PA) and provided free-choice acidified (pH 2.5) water in a polysulfone bottle with a neoprene stopper (Thoren Caging Systems) and irradiated feed (PicoLab Diet 5053, Purina, St Louis, MO). Two pieces of steam-sterilized, compressed, cotton nesting material (0.5 in.²; Nestlets, Ancare, Bellmore, NY) were

supplied in each cage during weekly changes in a horizontal-flow mass air-displacement unit (NU301, Nuair, Plymouth, MN). The holding room was ventilated with 100% outside filtered air at 10 to 15 air changes hourly. Temperature was maintained at 72 ± 2 °F (21.5 ± 1 °C), and relative humidity was maintained between 30% and 70%.

The affected colony, consisting of approximately 70 cages, was housed on one side of a 140-cage, double-sided, individually ventilated cage rack (9-140-10-14-1-4-5TM, Thoren Caging Systems) in an animal housing room containing 6 additional double-sided racks holding, in total, on average, 700 cages of unrelated mouse strains belonging to 3 investigative groups.

Colony health monitoring.

A cage of sentinel mice ($n = 4$, female Tac:SW, Taconic, Germantown, NY) on each double-sided rack that was exposed to soiled bedding from 40 cages (maintained on the associated rack) weekly at cage change and was tested bimonthly for ecto- and endoparasites and antibodies to mouse hepatitis virus, Sendai virus, Theiler mouse encephalomyelitis virus, pneumonia virus of mice, mouse parvovirus, mouse minute virus, lymphocytic choriomeningitis virus, epizootic diarrhea of infant mice virus, ectromelia virus, reovirus type 3 virus, K virus, mouse adenovirus, polyoma virus, cilia-associated respiratory bacillus, mouse cytomegalovirus, mouse thymic virus, Hantaan virus, *Mycoplasma pulmonis*, *Clostridium piliforme*, *Salmonella* spp., and *Citrobacter rodentium*.²³

Anatomic pathology.

Complete necropsies were performed on selected dead ($n = 3$) and clinically affected ($n = 7$) weanlings and on the 4 adult mice (affected dams; $n = 3$, asymptomatic dam; $n = 1$). The live mice were euthanized by CO₂ asphyxiation. Macroscopic lesions were recorded, tissues were fixed by immersion in 10% neutral buffered formalin, bones were decalcified in formic acid prior to processing, and representative sections of the following tissues were processed, embedded in paraffin, sectioned at a 4- μ m thickness, and stained with hematoxylin and eosin: adrenals, brain, bulbourethral gland, cervix, epididymides, esophagus, gallbladder, heart, intestines (small and large), kidney, liver, lungs, mandibular lymph nodes, mesenteric lymph nodes, ovaries, pancreas, parathyroid, pituitary, preputial gland, prostate, salivary glands, seminal vesicle, spinal cord, spleen, sternum, stifle joint (femur, tibia, muscle), stomach, testes, thymus, thyroid, trachea, urinary bladder, uterus, vertebral column, and spinal cord. Partial necropsies (brain only) were performed on additional clinically affected weanlings ($n = 7$). In addition, selected tissue sections were Gram-stained. All tissues were examined by a board-certified veterinary pathologist, and lesions were recorded.

Clinical pathology.

CBC analysis was performed on blood obtained from the index case and from an 86-d-old mouse from the same colony that presented with similar neurologic signs and had microscopically confirmed meningoencephalitis. The blood underwent automated assessment (Procyte Dx Hematology Analyzer, IDEXX Laboratories, Westbrook, ME). Blood smears were prepared and stained with Wright–Giemsa Romanowsky stain (Accustain Wright–Giemsa Stain Modified, Sigma-Aldrich, St. Louis, MO), and a manual WBC differential count was performed.

Bacteriology.

For 1 y after the presentation of the index cases, samples for aerobic and anaerobic bacteriologic culture were collected at necropsy ([Table 1](#)). In addition, the oral cavity ($n = 6$), rectum ($n = 5$), brain ($n = 3$), spleen ($n = 3$), uterus ($n = 3$), and vaginal cavity ($n = 3$) of timed-pregnant dams ($n = 9$) that either aborted ($n = 2$) or had offspring that died before weaning ($n = 7$); the oral cavity and rectum of sentinel animals on the affected rack ($n = 3$); the oral cavity of sentinel mice from additional racks ($n = 3$) in the room; the oral cavity of randomly selected mice from the same colony ($n = 5$); the oropharynx, nasopharynx, and palms of select employees ($n = 3$) with close and repeated contact with the affected animals; and the behavioral apparatus used in the associated studies were cultured.

Animals, personnel, and the behavioral apparatus were sampled by using sterile rayon-tipped swabs moistened with Modified Stuart Medium (BactiSwab NPG swabs, Remel, Lenexa, KS). Samples were plated onto sheep blood, chocolate, Columbia, Hektoen enteric, and MacConkey agar plates (Becton Dickinson, Franklin Lakes, NJ). Plates were incubated at 37 °C under anaerobic and aerobic conditions with 5% CO₂ for 72 h. In addition, blood, meninges, kidney, and spleen samples were plated under anaerobic conditions on *Brucella* plates. Pure cultures of the isolates were Gram-stained and a catalase test performed. The organisms then were characterized biochemically by using both the API 20 Strep and Rapid ID 32 Strep systems (BioMérieux, Marcy l'Etoile, France). Because of the inconsistencies associated with speciating this isolate, selected isolates ($n = 4$) were evaluated for growth in 6.5% NaCl (BBL Brain Heart Infusion broth with 6.5% NaCl, Becton Dickinson) at 10 °C and 45 °C. Antibiotic sensitivity was performed on each isolate ($n = 5$) by using the Kirby–Bauer disk diffusion technique.¹

One isolate collected from the meninges of a clinically affected weanling (mouse 12-5291) was sent to a reference laboratory (Clinical Microbiology Services, Columbia University Medical Center, New York, NY) for evaluation by using 2 automated bacterial identification systems (MicroScan Dried Conventional Gram Positive Panel, Panel 34, Siemens, Tarrytown, NY, and Vitek 2 GP-ID System, Vitek BioMérieux, France).

In an attempt to determine the source of this bacterium, timed-pregnant B6 mice from the 4 vendor breeding sites from which dams delivering offspring were known to have originated (site

1, $n = 6$; site 2, $n = 6$; site 3, $n = 6$; and site 4, $n = 5$) were cultured as described earlier immediately upon receipt ([Table 1](#)). Mice yielding positive cultures ($n = 1$) were maintained for 1 mo and then were euthanized for meningeal, oral, vaginal, rectal, uterine, and splenic cultures.

The oral cavities of additional mice (3 to 4 wk old, $n = 4$; female breeders, $n = 4$) at the vendor's breeding site were sampled for culture, as described earlier. These mice came from breeding site 4, the site from which we received the greatest number of affected animals.

Electron microscopy.

A bacterial pellet isolated from the meninges of a 29-d-old symptomatic weanling was fixed in 2.5% glutaraldehyde, 2% paraformaldehyde in 0.075 M sodium cacodylate buffer (Electron Microscopy Sciences, Hatfield, PA) for 1 h. The fixed pellet then was rinsed in the buffer and postfixed in osmium tetroxide for 1 h. Samples were rinsed in double-distilled water, dehydrated in a graded series of alcohol (50%, 75%, 95%, absolute alcohol) followed by propylene oxide, and overnight in 1:1 propylene oxide:PolyBed 812 (Polysciences, Warrington, PA). Ultra-thin sections were obtained (Reichert Ultracut S microtome, Leica Microsystems, Buffalo Grove, IL). Sections were stained with uranyl acetate and lead citrate and photographed by using a transmission electron microscope (1200 Ex, Jeol USA, Peabody, MA).

Bacterial 16S rRNA PCR sequencing and analysis.

Pure bacterial cultures isolated from the meninges of symptomatic weanlings ($n = 2$; mice 12-5291 and 13-1338) and the oral cavity of timed-pregnant dams ($n = 2$; clinically affected [mouse 12-5202] and unaffected [mouse 13-2043]), each with a distinct API biotype, were evaluated by PCR analysis by at least 1 of 3 laboratories and by using 3 unique generic bacterial 16S rRNA primer sets that amplified various portions of the 16S rRNA gene (*Escherichia coli* positions 911 to 1390, IDEXX Research Animal Diagnostic Laboratory, Columbia, MO; *E. coli* positions 5 to 531, Charles River Research Diagnostic Services; and *E. coli* positions 9 to 1541, Clinical Microbiology Services, Columbia University Medical Center; [Table 2](#)). Each laboratory sequenced the amplicons and compared the results with sequences in GenBank by using BLAST or proprietary software (AccuGENX-ID BacSeq, Charles River Research Diagnostic Services).

In addition, an oral cavity isolate from a 3- to 4-wk-old C57BL/6NCrI weanling (at the vendor's colony, mouse 39318) was evaluated by PCR assay using one of the aforementioned primer sets. We compared the sequence of the amplicon with those generated from the previously evaluated meningeal and oral isolates.

To definitively identify the bacterium, the entire 16S rRNA gene from 2 meningeal sites (animals 12-5291 and 13-1456) and an oral isolate from a symptomatic dam (12-5202) underwent full 16S rRNA sequencing (*E. coli* positions 9 to 1541). Genomic DNA from the bacterial strains was PCR-amplified by using 16S rRNA primers F24, 5' GAG TTT GAT YMT

GGC TCA G 3' (nt9 to 27, forward), and Y36, 5' GAA GGA GGT GWT CCA DCC 3' (nt1525 to 1541, reverse), as previously described.⁷ The full sequence was determined on both strands by using 6 primers (Table 2).³ The primers have been optimized for commercial Sanger sequencing at 50 °C, and sequencing was performed by Macrogen (Cambridge, MA). The 16S rRNA sequences for the mouse streptococcal strains examined in this study have been deposited into GenBank under accession numbers [KM609118](#) through [KM609123](#).

Construction of a phylogenetic tree.

The evolutionary history was inferred by using the neighbor-joining method.³³ The evolutionary distances were computed by using the Jukes–Cantor method.²⁰ The analysis was performed using the program RNA as previously described.⁶

Results

All ($n = 15$) symptomatic 23- to 30-d-old mice from the single affected colony were smaller than their unaffected littermates and were lethargic with a hunched posture (Table 3). Some symptomatic weanlings presented with an abnormal hopping gait ($n = 12$) and seizures ($n = 1$). Of the dams with clinical signs ($n = 2$), one was lethargic and in dystocia with a neonate lodged in the birth canal; the other was moribund and tachypneic with a litter of 21-d-old pups. In addition, a single 86-d-old male mouse presented with ruffled fur, hunched posture, lethargy, and tachypnea.

Pathology.

Of the 15 weanlings that presented with neurologic signs, 4 had focal or multifocal areas of superficial hemorrhage on the dorsocaudal aspect of the cerebral cortex (Figure 1, Table 3). All dead or symptomatic weanlings that were submitted for complete or partial necropsy ($n = 17$) had multifocal necrosuppurative and hemorrhagic meningoencephalitis and ventriculitis with intralesional gram-positive cocci (Figure 2). Marked lymphoid depletion of the thymus, lymph nodes, and spleen was present in 9 of the 10 cases that were submitted for complete necropsy. Less frequent lesions included mild to moderate multifocal suppurative pyelonephritis with numerous intralesional gram-positive cocci ($n = 2$), mild multifocal neutrophilic vasculitis in the soft tissues of the tongue ($n = 3$) and mandible ($n = 1$), moderate suppurative arthritis ($n = 1$), myeloid hyperplasia ($n = 12$), mild perivascular neutrophilic cellulitis of the salivary gland ($n = 1$), minimal to mild multifocal meningitis and gliosis of the spinal cord ($n = 3$), thymic necrosis ($n = 3$), mild multifocal subacute neutrophilic–lymphocytic and histiocytic glossitis ($n = 1$), and unilateral acute suppurative otitis interna ($n = 3$; Figure 3).

Clinical pathology.

The index case had a neutrophilia (4623 cells per μL) with slight toxic changes, eosinopenia (0 cells per μL), monocytosis (1449 cells per μL), and lymphopenia (759 cells per μL). The affected 86-d-old mouse had similar results on hematology.

Bacteriology.

An α -hemolytic, catalase-negative, gram-positive coccus with cells occurring in pairs or long chains was isolated from the meninges ($n=18$), kidney ($n=1$), oral cavity ($n=4$), uterus ($n=1$) and blood ($n=1$) from 21 of 68 cultured mice, 15 of which exhibited neurologic signs. Of the 21 mice with growth-positive cultures, 16 were weanlings (age, 23 to 30 d), 4 were timed-pregnant dams (2 clinically affected, one whose offspring died before weaning, and one asymptomatic), and 1 was an 86-d-old male mouse. This organism was the only bacterium isolated from the meninges, kidney, brain, blood, oral cavity, and uterus of affected animals. The same bacterium was isolated from the oral cavity of a timed-pregnant mouse that was cultured immediately on arrival from the vendor (asymptomatic dam; mouse 13-2043); we were unable to isolate the organism from the oral cavity, brain, spleen, uterus, or vaginal cavity from the same dam at necropsy 1 mo later. The bacterium was not isolated from asymptomatic littermates, randomly selected mice in the affected room, personnel in close contact with affected mice, the behavioral apparatus, or sentinel animals. The bacterium was isolated from the oral cavity of all 8 mice cultured at the vendor's breeding facility.

The bacterium grew readily on sheep blood, chocolate, and Columbia blood agars at 45 °C. The colonies (diameter, 1 mm) were gray-white and convex, with α -hemolysis visible at 48 h on sheep blood agar. No growth was seen in the brain heart infusion broth with 6.5% NaCl at 10 °C or on Hektoen enteric or MacConkey agar plates. Antibiotic sensitivity consistently demonstrated susceptibility to all common classes of antibiotics except trimethoprim-sulfamethoxazole.

The API 20 Strep system consistently identified 2 bacterial profiles (*Aerococcus viridans* 2 and 3; probabilities ranging from 55.7% to 98.3%) associated with 7 biotypes (7550510, 6750550, 7000551, 7000550, 6000550, 6550510, and 7510510); *S. acidominimus* was identified at low probability (43%). The Rapid ID 32 Strep system failed to identify the bacteria at high probability. The bacterium failed to produce acid from L-arabinose, D-ribose, D-sorbitol, inulin, glycogen, and starch. No activity was detected for acetoin (Voges-Proskauer test), 6-bromo-2-naphthyl- α -D-galactopyranoside, 2-naphthyl phosphate, and L-arginine. Isolates showed variable activity for pyroglutamic acid- β -naphthylamide, naphthol ASBI-glucuronic acid, and L-leucine- β -naphthylamide. All isolates hydrolyzed hippuric acid and esculin ferric citrate. One of the automated bacterial isolation systems (MicroScan Dried Conventional Gram-Positive Panel, Siemens) identified the isolate from the initial case (mouse 12-5291) as *S. mutans* with 99.78% confidence, whereas the second system (Vitek 2 GP-ID system, Vitek) could not identify the isolate.

Electron microscopy.

Cells were coccoid and 550 to 600 nm in diameter. Protein fibrils (fimbriae) extended from the thick cell-wall surface, which surrounded a dark-staining cell interior. Ultrastructural features were characteristic of *Streptococcus* species¹⁷ (Figure 4).

Bacterial 16S rRNA PCR sequencing and analysis.

None of the partial or complete 16S rRNA sequences generated from the meningeal and oral isolates were 100% homologous with the sequences for any named bacteria or clones in GenBank (Table 2). The partial 16S rRNA sequence from 2 samples (mice 12-5202 and 12-5291) displayed highest identity to *S. gallinaceus*. However, the short partial sequences were more than 99% identical to more than 155 *Streptococcus* sequences in GenBank, because the sequenced region (*E. coli* positions 911 to 1390) has high sequence similarity to several streptococcal species in this region of the 16S rRNA. By using a different partial 16S RNA primer set, the oral isolate from a breeding female housed in the vendor's colony (39318) and 2 isolates from symptomatic animals (nos. 13-2043 and 13-1338) were shown to be identical to one another and were most homologous to *S. hyointestinalis*, *S. sanguinis*, *S. salivarius*, *S. mitis*, and other *Streptococcus* species, at about 93% identity. Full 16S rRNA sequencing performed by using a third primer set (*E. coli* positions 9 to 1541) identified the isolate from mouse 12-5202 as being most similar to *S. acidominimus* (96.5% match). Additional full sequencing verified the sequence of the isolate from mouse 12-5202 and obtained sequences for the strains from mice 12-5291, 13-2043, 13-1338, and 13-1456. These 5 sequences were essentially identical (there is a single-nucleotide difference between the sequences from mouse 12-5202 and the other strains).

Figure 5 is a 16S rRNA based neighbor-joining tree for selected *Streptococcus* species, with *Enterococcus* species serving as an outgroup. The 3 novel *S. azizii* sequences are most closely related to *S. acidominimus*. Three 16S rRNA clone sequences in GenBank (clone COI-45 shown in the tree) from mouse sources cluster with *S. acidominimus*. The *S. azizii* sequences are next most closely related to a cluster of sequences including a strain from Taconic (*Streptococcus* sp. AHSI00047) and a clone from Smarlab (*Streptococcus* sp. Smarlab 3301444). The majority of sequences from this cluster are from isolates in mice, with a few isolated from human samples. Another neighboring cluster of sequences comes from rat cecum. These 4 taxa appear to represent a rodent clade of organisms within the streptococci.

Discussion

We identified a novel α -hemolytic gram-positive bacterium (*Streptococcus azizii* sp. nov.) from numerous weanling mice, presenting with runting, neurologic signs, and mortality, delivered by timed-pregnant dams; from 2 clinically affected timed-pregnant dams; 2 asymptomatic timed-

pregnant dams (one of which lost her litter before weaning); and an adult male mouse procured from a commercial vendor whose colony was enzootically infected. In mice presenting with neurologic signs, the bacterium was consistently associated with a diffuse necrosuppurative meningoencephalitis and ventriculitis in addition to lesions indicative of septicemia.

The bacterium could not be identified definitively by using biochemical techniques. The API Strep 20 system consistently identified the α -hemolytic cocci as *Aerococcus viridans*, a rarely reported human pathogen,³⁹ whereas 1 of the 2 automated bacterial identification systems identified our isolate as being most similar to *S. mutans*. In addition to these discrepancies, several other characteristics of the isolate were inconsistent with *Aerococcus* species.

It is well recognized that gram-positive, catalase-negative cocci, such as *Streptococcus* and *Aerococcus*, are challenging to speciate and differentiate from each other by using conventional microbiologic methods.^{14,19,32} Characteristic of streptococci, our isolates formed long chains or pairs after growth in broth or on agar, whereas *A. viridans* forms a characteristic tetrad when examined microscopically.³⁹ In addition, *Aerococcus* species can grow in 6.5% NaCl, whereas our isolate did not.³² To aid in identification, partial 16S rRNA sequencing was undertaken, which consistently confirmed the bacterium as a *Streptococcus* species. Partial sequence analysis led to inconsistencies in species identification. The complete 16S rRNA sequence confirmed the isolate to be a unique *Streptococcus*, most closely related to but distinct from *S. acidominimus* strain LGM 17755 and *Streptococcus* sp. Smarlab 3301444. The partial sequences were identical to the corresponding sections of the full sequences but demonstrated the difficulties of trying to use partial sequences for identifying a potentially novel organism. We have designated this novel species as *S. azizii* sp. nov.

α -Hemolytic *Streptococcus* species are characteristically hard to speciate by standard microbiologic diagnostics.^{14,19,32} They do not fall within the typical serologic Lancefield groups, as do β -hemolytic *Streptococcus* species. The classification of α -hemolytic *Streptococcus* spp. remains under debate, accounting for the difficulty we had in identifying this isolate by both biochemical and molecular methods.⁹

Streptococcus species typically are commensals that inhabit the nose, mouth, intestine, genital tract, and skin of mammals.² In mice, pathogenic streptococci known to affect both immunocompetent and immunocompromised strains are generally β -hemolytic and are associated with suppurative renal, cardiac, splenic, hepatic, thoracic, uterine, lymph node, and pulmonary lesions.^{2,31}

There are 2 documented case series associated with α -hemolytic *Streptococcus* species that resulted in clinical disease in immunodeficient mice.³⁰ The first, associated with *S. viridans*, occurred in irradiated SCID and SCID/beige mice with splenomegaly, pulmonary congestion, and septicemia.³⁰ In the second series, which involved increased mortality in

immunocompromised strains maintained by a commercial vendor, a gram-positive, α -hemolytic cocci (<http://www.ncbi.nlm.nih.gov/nuccore/KM609123.1>) was isolated from the blood, oral, and vaginal cultures of several moribund mice. These mice had a subacute meningitis and meningoencephalitis, endocarditis, nephritis, and oophoritis. The causative bacterium was identified to the *Streptococcus* genus in the *S. viridans* group. Viridans streptococci are generally known as the ‘leftovers’ of the *Streptococcus* species, and their taxonomic classification is controversial.⁹ A comparison of the full 16S rRNA sequence of our isolates with that obtained from the second case series (*Streptococcus* sp. AHSI00047) reveals that they are distinct species.

Routine monitoring for β -hemolytic *Streptococcus* species is recommended by the Federation of European Laboratory Animal Science Associations and is commonly performed by large commercial breeders in the United States.²⁴ However, given that there are only 3 manuscripts describing disease associated with naturally occurring α -hemolytic *Streptococcus* species (including this manuscript), routine monitoring for α -hemolytic *Streptococcus* species is not currently recommended by the Federation.²⁴

Figure 4 shows 5 nearly identical sequences that fall into this unnamed taxa. The Smarlab 3301444 strain from a human clinical sample is grouped with strains from mouse trachea, mouse lung and mouse meninges. A single human skin clone shown in the tree represents 10 clones from a human skin library (from GenBank entries, subject HV2). The exposure of subject HV2 to mice or other animals was not recorded.

The precipitating factors that led to the significant morbidity and mortality in these immunocompetent weanling B6 mice remains unknown. Of note, all affected weanlings were born to dams that were transported during days 17 to 18 of gestation. Animal transport significantly affects adult mammalian physiology.^{4,5,8,10,15,21,28,38} For example, plasma corticosterone concentration significantly increased in mice immediately after inhouse transport, which presumably is less stressful than is ground transport over considerably longer distances.⁵ In deer mice, *Peromyscus maniculatus*, reproduction was suppressed for several weeks after transatlantic shipping.¹⁵ The effects of transport stress are well-documented in livestock and NHPs as well.^{5,10,21,28,36-38} Transport stress during gestation could have predisposed dams to shed the bacterium, infecting their offspring, or, as we observed, to develop clinical disease.

Maternal stress, such as that occurring during shipping, can affect the developing fetus.^{11,26,29} Mice and rats subject to prenatal stress have consistently been shown to have elevated glucocorticoids, alterations in hypothalamic–pituitary–adrenal axis programming, altered structural and functional neuroanatomy, and changes in innate and reactive behavioral profiles.^{11,29} In addition, maternal stress in rodents has been shown to have a negative effect on the reproductive performance of offspring for at least one generation.¹⁴ Prenatal stress on the neonatal immune system is generally immunosuppressive. Fetal mice exposed to stress during the last few days of gestation can have inhibited macrophage and neutrophil function until they

reach 2 mo of age.²⁹ In our mice, almost all of the symptomatic weanlings that underwent complete microscopic examination showed thymic lymphoid depletion, which can be caused by stress and leads to a compromised immune system.^{8,18}

The mouse immune system is poorly developed until approximately day 30, with the establishment of immune memory not occurring until between 30 and 60 d of age, thus increasing the susceptibility to opportunistic infection at weaning.²² Maternal IgG levels peak at postnatal day 16 around the time of gut closure and then rapidly drop until day 28, when there is a period of transient hypogammaglobulinemia. Although IgM levels begin to rise around 3 wk of age, adult levels are not reached until 6 mo of age.²²

We speculate that the combination of transport stress, declining maternal antibody, weanling immune incompetence, and the presence of this opportunistic bacterium transmitted from either the dam's oral cavity or urogenital tract (or both) during or after parturition led to neonatal infection and clinical disease. It remains unclear why some mice remained uninfected and others developed severe clinical signs. In addition, there appears to be a potential for an animal to be transiently infected, given that the only naïve dam from which we obtained a positive oral culture on arrival was culture-negative 1 mo later.

We have not completely elucidated the pathogenesis of this novel *Streptococcus* species. There are 2 *Streptococcus* species that commonly cause meningitis, *S. pneumoniae* and *S. suis*.^{25,34,35} The pathogenesis of both bacteria is poorly understood; however, several hypotheses have been proposed.^{13,27} The bacterium is proposed to enter the bloodstream after transversing the oral cavity mucosa, presumably with the aid of virulence factors. For *S. suis* at least, the bacterium is speculated to then bind to or be engulfed by macrophages, leading to a persistent bacteremia. Finally, the bacteria crosses the blood–brain barrier, although, as with *S. azizii*, it remains to be elucidated why the meninges remain a site of predilection for these types of *Streptococcus* spp. A ‘Trojan Horse’ theory, in which the bacteria enter the brain in monocytes, has also been proposed in addition to free bacterial interaction with the blood–brain barrier. Because *S. azizii* has similar characteristics to these *Streptococcus* spp., we suspect its pathogenesis may be similar as well.

Because of the nature of the research in which they were involved, we elected not to treat the mice with antibiotics, which we speculate would be effective when administered preemptively or early in the disease course. Researchers requiring late-gestation, timed-pregnant C57BL/6NCrl mice should be aware of the possibility of infection and disease in weanling mice and may benefit from procuring animals from colonies free of this bacterium or from using antimicrobials in face of disease. Decreasing maternal and pup stress during and after transport may be beneficial also.

In conclusion, we describe the first documented cases of a unique opportunistic α -hemolytic *Streptococcus* species (*Streptococcus azizii* sp. nov.) that was associated with weanling morbidity and mortality in a commonly used immunocompetent mouse strain. Experimental studies likely will shed light on the pathogenesis and pathogenicity of this organism.

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References

1. Bauer AW, Kirby WM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single-disk method. *Am J Clin Pathol* 45:493–496. [[PubMed](#)]
2. Besch-Williford C, Franklin CL. 2007. Aerobic gram-positive organisms, p 389–406. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith A, editors. *The mouse in biomedical research*, 2nd ed Waltham (MA): Academic Press
3. Camanocha A, Dewhirst FE. 2014. Host-associated bacterial taxa from Chlorobi, Chloroflexi, GN02, Synergistetes, SR1, TM7, and WPS2 phyla or candidate divisions. *J Oral Microbiol*.6.[[PMC free article](#)] [[PubMed](#)]
4. Castelhana-Carlos MJ, Baumans V. 2009. The impact of light, noise, cage cleaning and inhouse transport on welfare and stress of laboratory rats. *Lab Anim* 43:311–327. [[PubMed](#)]
5. Chirase NK, Greene LW, Purdy CW, Loan RW, Auvermann BW, Parker DB, Walborg EF, Jr, Stevenson DE, Xu Y, Klaunig JE. 2004. Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. *Am J Vet Res* 65:860–864. [[PubMed](#)]
6. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, Lakshmanan A, Wade WG. 2010. The human oral microbiome. *J Bacteriol* 192:5002–5017. [[PMC free article](#)] [[PubMed](#)]
7. Dewhirst FE, Klein EA, Thompson EC, Blanton JM, Chen T, Milella L, Buckley CM, Davis IJ, Bennett ML, Marshall-Jones ZV. 2012. The canine oral microbiome. *PLoS ONE* 7:e36067.[[PMC free article](#)] [[PubMed](#)]

8. Drozdowicz CK, Bowman TA, Webb ML, Lang CM. 1990. Effect of inhouse transport on murine plasma corticosterone concentration and blood lymphocyte populations. *Am J Vet Res* 51:1841–1846. [[PubMed](#)]
9. Facklam R. 2002. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev* 15:613–630. [[PMC free article](#)] [[PubMed](#)]
10. Fernstrom AL, Sutian W, Royo F, Westlund K, Nilsson T, Carlsson HE, Paramastri Y, Pamungkas J, Sajuthi D, Schapiro SJ, Hau J. 2008. Stress in cynomolgus monkeys (*Macaca fascicularis*) subjected to long-distance transport and simulated transport housing conditions. *Stress* 11:467–476. [[PubMed](#)]
11. Fonseca ES, Massoco CO, Palermo-Neto J. 2002. Effects of prenatal stress on stress-induced changes in behavior and macrophage activity of mice. *Physiol Behav* 77:205–215. [[PubMed](#)]
12. Garnier F, Gerbaud G, Courvalin P, Galimand M. 1997. Identification of clinically relevant viridans group streptococci to the species level by PCR. *J Clin Micro* 35:2337–2341. [[PMC free article](#)] [[PubMed](#)]
13. Gottschalk M, Segura M. 2000. The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. *Vet Microbiol* 76:259–272. [[PubMed](#)]
14. Haanpera M, Jalava J, Huovinen P, Meurman O, Rantakokko-Jalava K. 2007. Identification of α -hemolytic streptococci by pyrosequencing the 16S rRNA gene and by use of Vitek 2. *J Clin Microbiol* 45:762–770. [[PMC free article](#)] [[PubMed](#)]
15. Hayssen V. 1998. Effect of transatlantic transport on reproduction of agouti and nonagouti deer mice, *Peromyscus maniculatus*. *Lab Anim* 32:55–64. [[PubMed](#)]
16. Hedrich HJ, Bullock GR, Petrusz P. 2004. The laboratory mouse. Amsterdam (NL): Elsevier Academic Press.
17. Holt SC, Leadbetter ER. 1976. Comparative ultrastructure of selected oral streptococci: thin-sectioning and freeze-etching studies. *Can J Microbiol* 22:475–485. [[PubMed](#)]
18. Huff GR, Huff WE, Balog JM, Rath NC, Anthony NB, Nestor KE. 2005. Stress response differences and disease susceptibility reflected by heterophil to lymphocyte ratio in turkeys selected for increased body weight. *Poult Sci* 84:709–717. [[PubMed](#)]
19. Janda JM, Abbott SL. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J Clin Microbiol* 45:2761–2764. [[PMC free article](#)][[PubMed](#)]
20. Jukes TH, Cantor CR. 1969. Evolution of protein molecules. New York (NY): Academic Press.

21. Kim CY, Han JS, Suzuki T, Han SS. 2005. Indirect indicator of transport stress in hematological values in newly acquired cynomolgus monkeys. *J Med Primatol* 34:188–192. [[PubMed](#)]
22. Landreth KS. 2002. Critical windows in development of the rodent immune system. *Hum Exp Toxicol* 21:493–498. [[PubMed](#)]
23. Lipman NS, Homberger FR. 2003. Rodent quality-assurance testing: use of sentinel animal systems. *Lab Anim (NY)* 32:36–43. [[PubMed](#)]
24. Mahler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M. 2014. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. *Lab Anim* 48:178–192. [[PubMed](#)]
25. Medina E. 2010. Murine model of pneumococcal pneumonia. *Methods Mol Biol* 602:405–410. [[PubMed](#)]
26. Merlot E, Quesnel H, Prunier A. 2013. Prenatal stress, immunity, and neonatal health in farm-animal species. *Animal* 7:2016–2025. [[PubMed](#)]
27. Mook-Kanamori BB, Geldhoff M, van der Poll T, van de Beek D. 2011. Pathogenesis and pathophysiology of pneumococcal meningitis. *Clin Microbiol Rev* 24:557–591. [[PMC free article](#)][[PubMed](#)]
28. Mota-Rojas D, Becerril-Herrera M, Roldan-Santiago P, Alonso-Spilsbury M, Flores-Peinado S, Ramirez-Necoechea R, Ramirez-Telles JA, Mora-Medina P, Perez M, Molina E, Soni E, Trujillo-Ortega ME. 2012. Effects of long-distance transportation and CO₂ stunning on critical blood values in pigs. *Meat Sci* 90:893–898. [[PubMed](#)]
29. Palermo Neto J, Massoco CO, Favare RC. 2001. Effects of maternal stress on anxiety levels, macrophage activity, and Ehrlich tumor growth. *Neurotoxicol Teratol* 23:497–507. [[PubMed](#)]
30. Percy DH, Barta JR. 1993. Spontaneous and experimental infections in scid and scid/beige mice. *Lab Anim Sci* 43:127–132. [[PubMed](#)]
31. Percy DH, Barthold SW. 2007. *Pathology of laboratory rodents and rabbits*. Ames (IA): Blackwell Publishing.
32. Ruoff KL. 2002. Miscellaneous catalase-negative, gram-positive cocci: emerging opportunists. *J Clin Microbiol* 40:1129–1133. [[PMC free article](#)] [[PubMed](#)]
33. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. [[PubMed](#)]

34. Schliefer KH. 2009. The firmicutes. In: Vos PD, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schliefer KH, Whitman W, editors. *Bergey's manual of systematic bacteriology*, vol 3. New York (NY): Springer.
35. Segura M, Zheng H, de Greeff A, Gao GF, Grenier D, Jiang Y, Lu C, Maskell D, Oishi K, Okura M, Osawa R, Schultsz C, Schwerk C, Sekizaki T, Smith H, Srimanote P, Takamatsu D, Tang J, Tenenbaum T, Tharavichitkul P, Hoa NT, Valentin-Weigand P, Wells JM, Wertheim H, Zhu B, Gottschalk M, Xu J. 2014. Latest developments on *Streptococcus suis*: an emerging zoonotic pathogen. Part 1. *Future Microbiol* 9:441–444. [[PubMed](#)]
36. Segura M, Zheng H, de Greeff A, Gao GF, Grenier D, Jiang Y, Lu C, Maskell D, Oishi K, Okura M, Osawa R, Schultsz C, Schwerk C, Sekizaki T, Smith H, Srimanote P, Takamatsu D, Tang J, Tenenbaum T, Tharavichitkul P, Hoa NT, Valentin-Weigand P, Wells JM, Wertheim H, Zhu B, Xu J, Gottschalk M. 2014. Latest developments on *Streptococcus suis*: an emerging zoonotic pathogen: Part 2. *Future Microbiol* 9:587–591. [[PubMed](#)]
37. Seidler T, Alter T, Kruger M, Fehlhaber K. 2001. Transport stress—consequences for bacterial translocation, endogenous contamination, and bactericidal activity of serum of slaughter pigs. *Berl Munch Tierarztl Wochenschr* 114:375–377. [[PubMed](#)]
38. Zhang L, Yue HY, Zhang HJ, Xu L, Wu SG, Yan HJ, Gong YS, Qi GH. 2009. Transport stress in broilers: I. Blood metabolism, glycolytic potential, and meat quality. *Poult Sci* 88:2033–2041. [[PubMed](#)]
39. Zhou W, Nanci V, Jean A, Salehi AH, Altuwaijri F, Cecere R, Genest J. 2013. *Aerococcus viridans* native valve endocarditis. *Can J Infect Dis Med Microbiol* 24:155–158. [[PMC free article](#)][[PubMed](#)]

Table 1.

Culture sites for bacteriology

	No. of samples from listed site							
	Meninges	Oral Cavity	Kidney	Spleen	Uterus	Blood	Rectum	Vagina
Weanlings (21 to 30 d old; <i>n</i> = 18)	18	3	2	15	NP	1	NP	NP
Affected adult mouse (86 d	1	1	NP	1	NP	NP	1	NP

No. of samples from listed site

	Meninges	Oral Cavity	Kidney	Spleen	Uterus	Blood	Rectum	Vagina
old; $n = 1$)								
Affected dams ($n = 2$)	2	1	NP	2	2	NP	1	1
Dams with affected litters ($n = 9$)	3	6	NP	3	3	NP	5	3
Sentinels ($n = 6$)	NP	6	NP	NP	NP	NP	NP	NP
Littermates of affected weanlings ($n = 4$)	NP	4	NP	NP	NP	NP	3	2
Random mice in room ($n = 5$)	NP	5	NP	NP	NP	NP	NP	NP
Timed-pregnant dams ($n = 23$) ^a	NP	23	NP	NP	NP	NP	NP	23

NP, not performed.

^aCultured on receipt

Table 2.

Bacterial isolates analyzed by partial or complete 16S rRNA sequencing

Isolate no.	Isolation site	Signalment	IDEXX-RES partial 16S rRNA sequence (nt 911 to 1390)	CRRDS partial 16S rRNA sequence (nt 5 to 531)	Columbia University complete 16S rRNA (nt 9 to 1541)	Forsyth Institute complete 16S rRNA (nt 8 to 1541)
12-5202	Oral cavity	Timed-pregnant dam; litter found dead	99.5% identity (429 of 431 nt)	NP	96.4% identity (1455 of 1503 nt) to <i>S.</i>	96.5% identity (1450 of 1503 nt) to <i>S. acidominimus</i> NR_104972 ; 96.3% identity (1455

Isolate no.	Isolation site	Signalment	IDEXX-RES partial 16S rRNA sequence (nt 911 to 1390)	CRRDS partial 16S rRNA sequence (nt 5 to 531)	Columbia University complete 16S rRNA (nt 9 to 1541)	Forsyth Institute complete 16S rRNA (nt 8 to 1541)
			to <i>S. gallinaceus</i> ; <99% identity to 155 GenBank entries		<i>acidominimus</i> ^a	of 1511 nt) to <i>Streptococcus</i> sp. Smarlab AY538685
12-5291	Meninges	29-d-old male found runted, hunched, with neurologic signs	99.5% identity (429 of 431 nt) to <i>S. gallinaceus</i> ; <99% identity to 155 GenBank entries	NP	NP	96.5% identity (1450 of 1503 nt) to <i>S. acidominimus</i> NR_104972 ; 96.3% identity (1455 of 1511 nt) to <i>Streptococcus</i> sp. Smarlab AY538685
13-2043	Oral cavity	Timed-pregnant dam, cultured on arrival	NP	93.4% identity (533 of 535 nt) to <i>S. hyointestinalis</i>	NP	96.5% identity (1450 of 1503) to <i>S. acidominimus</i> NR_104972 ; 96.3% identity (1455 of 1511 nt) to <i>Streptococcus</i> sp. Smarlab AY538685
13-1338	Meninges	25-d-old male found runted, hunched, with neurologic signs	NP	93.43% identity (533 of 535 nt) to <i>S. hyointestinalis</i>	NP	96.5% identity (1450 of 1503 nt) to <i>S. acidominimus</i> NR_104972 ; 96.3% identity (1455 of 1511 nt) to <i>Streptococcus</i> sp. Smarlab AY538685
13-	Oral	Timed-	NP	NP	NP	96.5% identity (1450 of

Isolate no.	Isolation site	Signalment	IDEXX-RES partial 16S rRNA sequence (nt 911 to 1390)	CRRDS partial 16S rRNA sequence (nt 5 to 531)	Columbia University complete 16S rRNA (nt 9 to 1541)	Forsyth Institute complete 16S rRNA (nt 8 to 1541)
1456	cavity	pregnant dam found moribund				1503 nt) to <i>S. acidominimus</i> NR_104972 ; 96.3% identity (1455 of 1511 nt) to <i>Streptococcus</i> sp. Smarlab AY538685
39318	Oral cavity	3- to 4-wk-old C57BL/6NCrl vendor colony mouse	NP	93.4% identity (533 of 535 nt) to <i>S. hyointestinalis</i>	NP	NP

NP, not performed.

^aIdentified by MicroScan Pos Combo 34 panel (Siemens) as *S. mutans* at a 99.79% confidence level

Table 3.

Clinical signs and results of histopathology and microbiology of affected mice

ID	Clinical signs						Histopathology				Microbiology			
	Ven site	Age	Found dead	Runting	Neurologic signs	Other	Meningo-encephalitis	Lymphoid depletion	Bacteremia-associated lesions	Meninges	Oral cavity	Kidney	Spleen	Other
Weanlings (<i>n</i> = 18)														
U0	3	0	-	+	-	-	+	+	+	NP	NP	NP	NP	NP
1	2	2	+	NA	NA	NA	+	+	+	+ ^g	NP	NP	NP	NP

ID	Ven dor site	A ge	Clinical signs				Histopathology			Microbiology				
			Fou nd dea d	Runt ing	Neurol ogic signs	Oth er	Mening o- enceph alitis	Lymp hoid deple tion	Bactere mia- associa ted lesions	Meni nges	Ora l cav ity	Kid ney	Sple en	Oth er
		6												
	1	30	-	+	-	-	+	+	+	+ ^g	NP	+	-	NP
	1	30	+	NA	NA	NA	+	+	+	+ ^g	NP	NP	NP	NP
	1	29	-	NA	NA	NA	+ ^e	+	+	+ ^g	NP	NP	NP	NP
12 - 52 01	1	29	-	+	+ ^a	-	+	-	-	+ ^{g,n,o}	NP	-	-	+ ^{g,p}
	U	30	-	+	+	-	NP	NP	NP	-	NP	NP	NP	NP
	U	28	-	+	+ ^a	-	+ ^e	+	+	+ ^{h,n}	NP	NP	-	NP
	U	28	-	+	+ ^a	-	+	+	+	+ ⁱ	NP	NP	-	NP
	1	28	-	+	+ ^a	-	+	+	+	+ ^j	NP	NP	-	NP
	1	26	-	+	+ ^a	-	+ ^{e,f}	NP	+	+ ^g	NP	NP	-	NP
	1	25	-	+	+ ^a	-	+ ^f	NP	+	+ ^k	NP	NP	-	NP
	1	2	-	+	+ ^a	-	+ ^{e,f}	NP	+	+ ^j	NP	NP	-	NP

ID	Ven dor site	A ge	Clinical signs				Histopathology				Microbiology			
			Fou nd dea d	Runt ing	Neurol ogic signs	Oth er	Mening o- enceph alitis	Lymp hoid deple tion	Bactere mia- associa ted lesions	Meni nges	Ora l cav ity	Kid ney	Sple en	Oth er
		5												
13 - 13 38	1	2 5	-	+	+ ^a	-	+ ^f	NP	+	+ ^{k,n}	NP	NP	-	NP
	1	2 3	-	+	+ ^a	-	+	+	+	+ ^k	NP	NP	-	NP
	1	2 9	-	+	+ ^a	-	+ ^f	NP	+	+ ^m	-	NP	-	- ^q
	1	2 9	-	+	+ ^a	-	+ ^f	NP	+	+ ^m	-	NP	-	- ^q
	1	2 9	-	+	+ ^a	-	+ ^f	NP	+	+ ^m	-	NP	-	- ^q
Dams (n = 4)														
	2	1 0 - 1 3 w k	-	-	-	+ ^b	-	+	+	-	NP	NP	-	+ ^{k,r}
12 - 52 91	2	1 0 - 1 3 w	-	-	-	+ ^c	-	-	-	-	+ ^{m,n}	NP	-	- ^r

ID	Ven dor site	A ge	Clinical signs				Histopathology				Microbiology			
			Fou nd dea d	Runt ing	Neurol ogic signs	Oth er	Mening o- enceph alitis	Lymp hoid deple tion	Bactere mia- associa ted lesions	Meni nges	Ora l cav ity	Kid ney	Sple en	Oth er
13 - 14 56	1	1 0 - 1 3 w k	-	-	-	+ ^d	+	-	+	+ ^m	+ ^l	NP	-	- q,r,s
13 - 20 43	2	1 0 - 1 3 w k	-	-	-	-	-	-	-	-	+ ^{k,n}	NP	-	- q,r,s
Adult (n = 1)														
	4	8 6 d	-	-	-	+ ^d	NP	NP	NP	+ ⁱ	+ ⁱ	NP	-	- ^q

NA, not applicable; NP, not performed; U, undetermined.

Clinical signs: ^aabnormal gait, ^bdystocia, ^clost litter, and ^dmoribund.

^eFocal cerebral hemorrhage.

^fPartial necropsy (head only).

API biotype ^g7550510, ^h6750550, ⁱ7000551, ^j7000550, ^k6000550, ^l6550510, and ^m751510.

Sample evaluated by ⁿPCR assay for *S. azizii* or ^oelectron microscopy.

Culture site: ^pblood, ^qrectum, ^ruterus, and ^svagina.

Figure 1- Focal area of hemorrhage on the caudodorsal aspect of the cerebral cortex in a 30-d-old mouse. Bar, 1 cm.



Figure 2. Photomicrograph of a coronal section of the cerebral cortex, hippocampus, lateral and third ventricles, and thalamus, with necrosuppurative meningoencephalitis and ventriculitis (short arrow) and meningeal hemorrhage (long arrow) Hematoxylin and eosin stain; bar, 500 μ m. Inset, Gram-stained section of ventricle with myriad intra- and extracellular gram-positive cocci. Bar, 20 μ m.

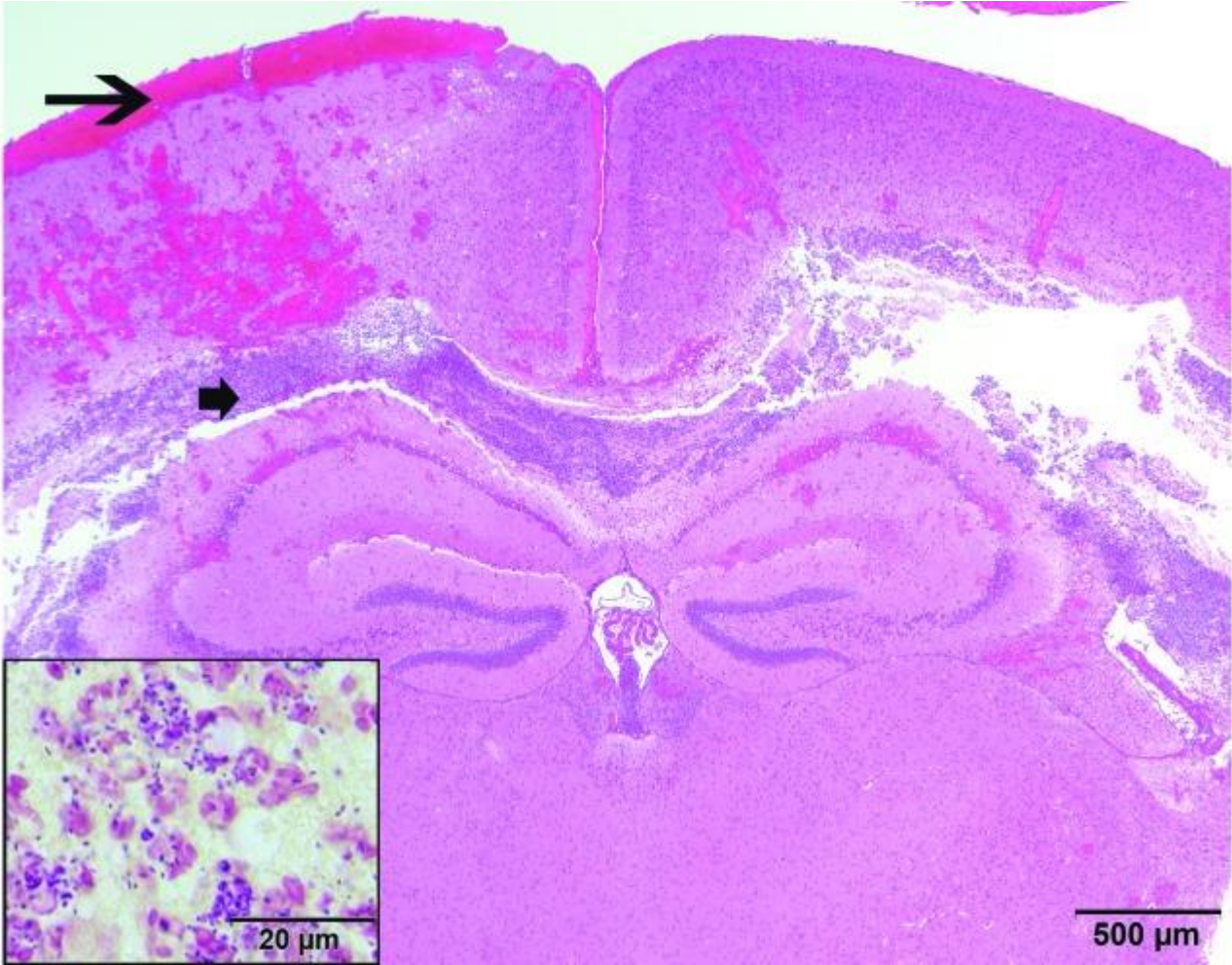


Figure 3. (A) Photomicrograph of a coronal section of the inner ear with large numbers of degenerate neutrophils (arrows). Bar, 100 μ m. (B) Frequent foci of necrosuppurative inflammation in the placenta and uterus (asterisk) with moderate numbers of coccobacillary bacteria along the luminal surface and the uterus–placenta interface (arrows). Bar, 50 μ m. (C) Marked cortical lymphoid depletion (arrows). Bar, 100 μ m. (D) Suppurative pyelonephritis in the kidney. Degenerate neutrophils (asterisk) and bacterial colonies (arrows) fill the renal tubules of the medulla. Bar, 50 μ m.

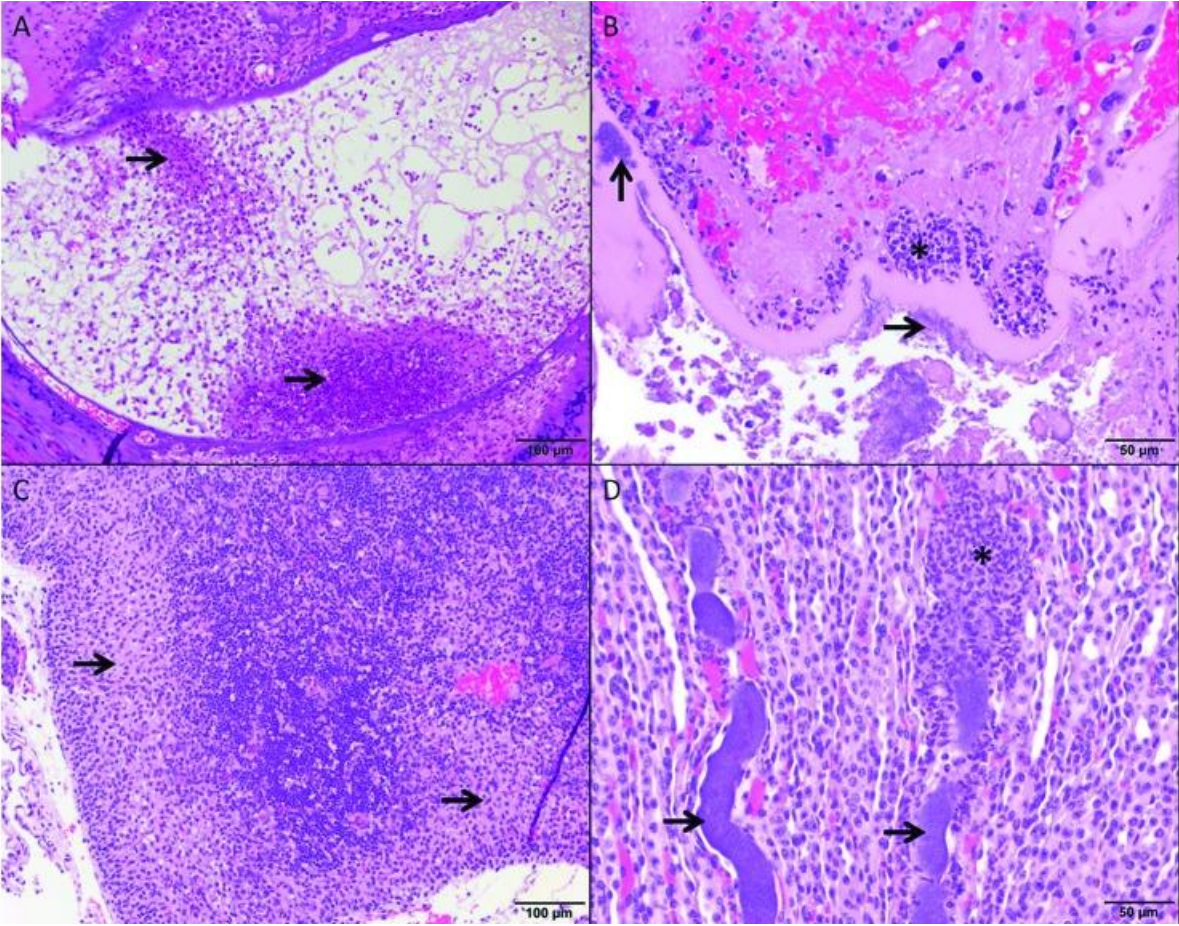


Figure 4. Transmission electron micrograph of *Streptococcus azizii*. Surface fibrils (short arrow) surround a thick electron-lucent cell wall, which surrounds the electron-dense cell interior. An electron-lucent septum (arrow) between dividing cells is present. Magnification, 75,000 \times .

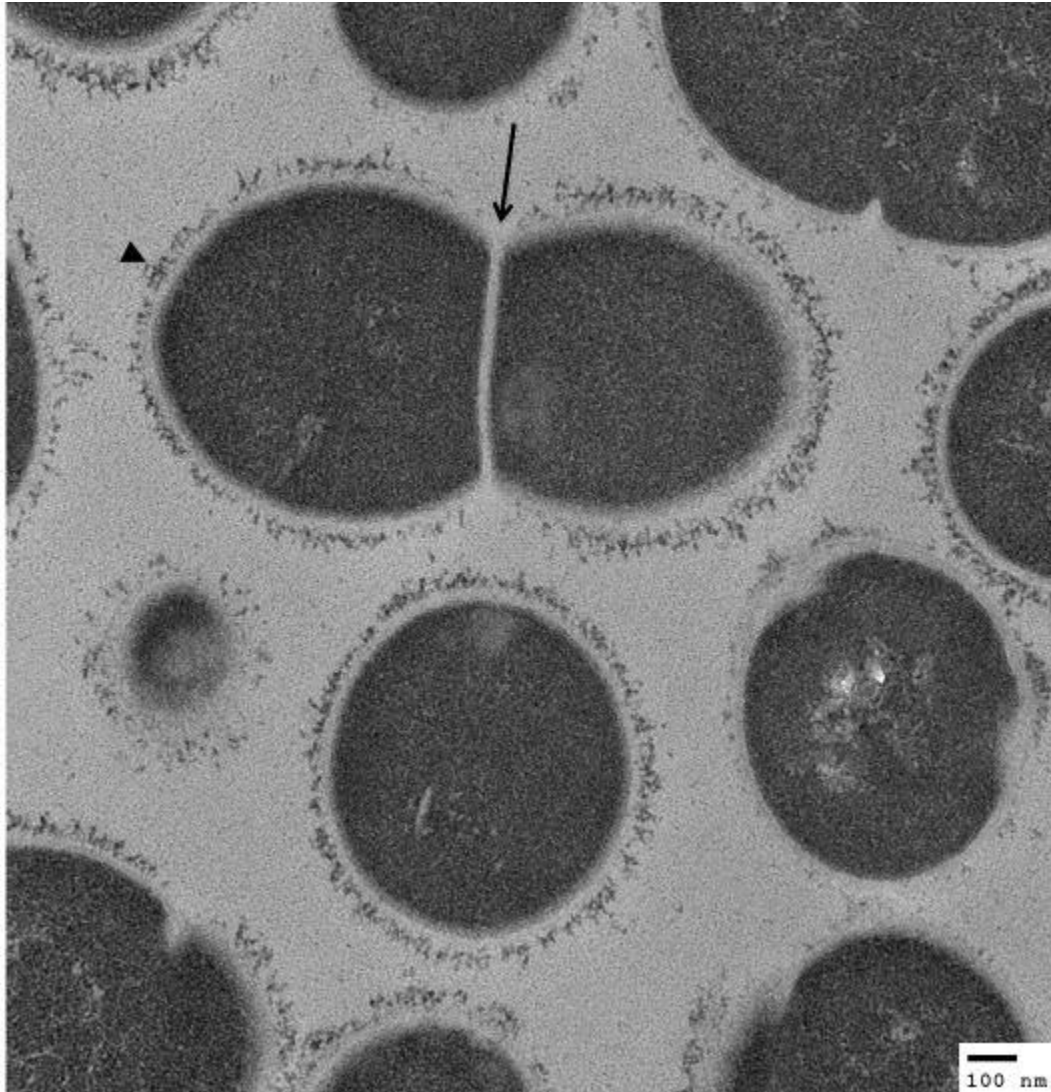


Figure 5. Phylogenetic tree for *Streptococcus azizii* sp. nov. and selected *Streptococcus* species.

