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Streptobacillus moniliformis and IgM and IgG Immune Response in Patient with Endocarditis[1]

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We describe a case of endocarditis caused by *Streptobacillus moniliformis* bacteria, a known cause of rat-bite fever, in a 32-year-old woman with pet rats in Germany. The patient had a strong serologic response, with high IgM and IgG titers. Serologic analysis is a promising tool to identify *S. moniliformis* bacterial infection.

Rat-bite fever (RBF) is a rare disease that typically manifests with fever, rash, and arthritis (*1*). Possible complications are abscess formation, endocarditis, and death if left untreated (*1*,*2*). *Streptobacillus moniliformis* bacteria is the main causative pathogen of RBF (*3*). Norway rats (*Rattus norvegicus*) are the natural host and usually carry *S. moniliformis* bacteria asymptomatically in their nasopharynx (*3*,*4*). Transmission occurs typically by rat bite or scratch but also by nontraumatic indirect contact.

We describe a case of a 32-year-old woman who came to an emergency department in Germany in May 2022 with fever, fatigue, and migrating arthralgia in the large and small joints of all 4 extremities, without signs of joint swelling or rash. She had a short history of diarrhea, and her first set of blood cultures were negative. She was initially diagnosed with reactive arthritis and transferred to the rheumatology department. We initiated treatment with 20 mg prednisolone and etoricoxib. The patient had initial relief of symptoms and was discharged after 6 days in the hospital. A small papule on her right foot

[1]Preliminary results from this study were presented at the 51st Congress of the German Rheumatological Society, August 30–September 2, 2023, Leipzig, Germany.

[2]These senior authors contributed equally to this article.

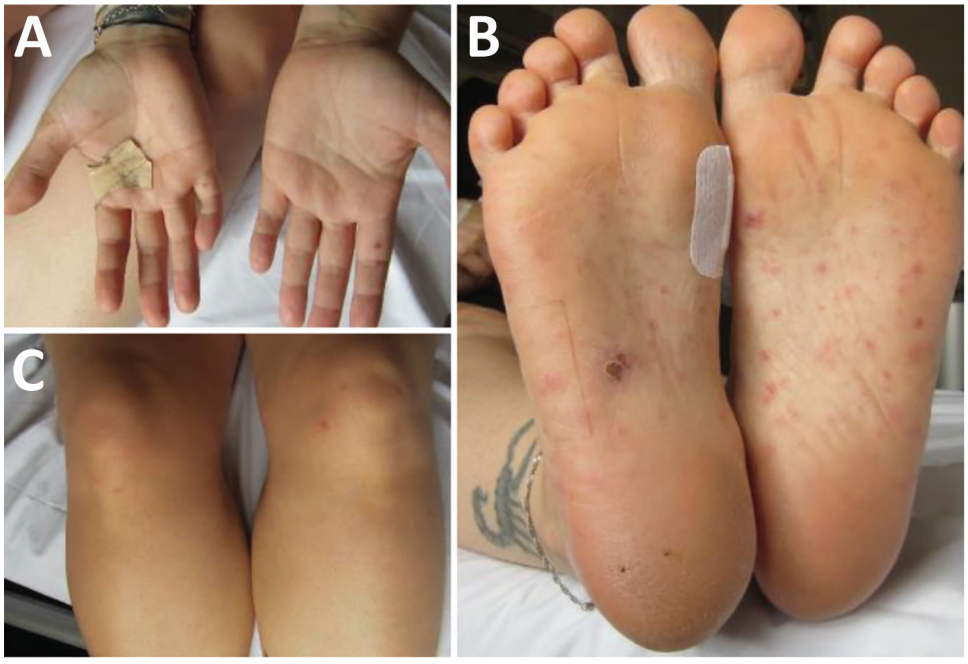

Figure 1. Rat bite fever lesions on 32-year-old female patient, Germany, 2022. At the time of patient's readmission, reddish papules appeared on the palms of the hands (A), soles of the feet (B), and legs (C).

appeared immediately after discharge. A few days later, she went to the dermatology department with a fever and red, nonitching papules on hands, legs, and feet (Figure 1). We examined the papules, finding them comparable to Janeway lesions, and took a biopsy from the right hand. We collected a second blood culture that was positive within 18 hours with growth of a gram-negative bacilli. We identified *S. moniliformis* bacteria by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

The patient was readmitted. In an extended history, she reported having 3 Norway rats as pets. Our further investigation revealed an 11-mm size vegetation on the right coronary cusp of the aortic valve; we observed no signs of insufficiency during echocardiography. The patient was diagnosed with RBF and probable aortic valve endocarditis because of meeting 1 major criterion (positive echocardiography) and 2–3 minor criteria (fever, positive blood culture, and suspected Janeway lesions) of the modified Duke criteria (*5*).

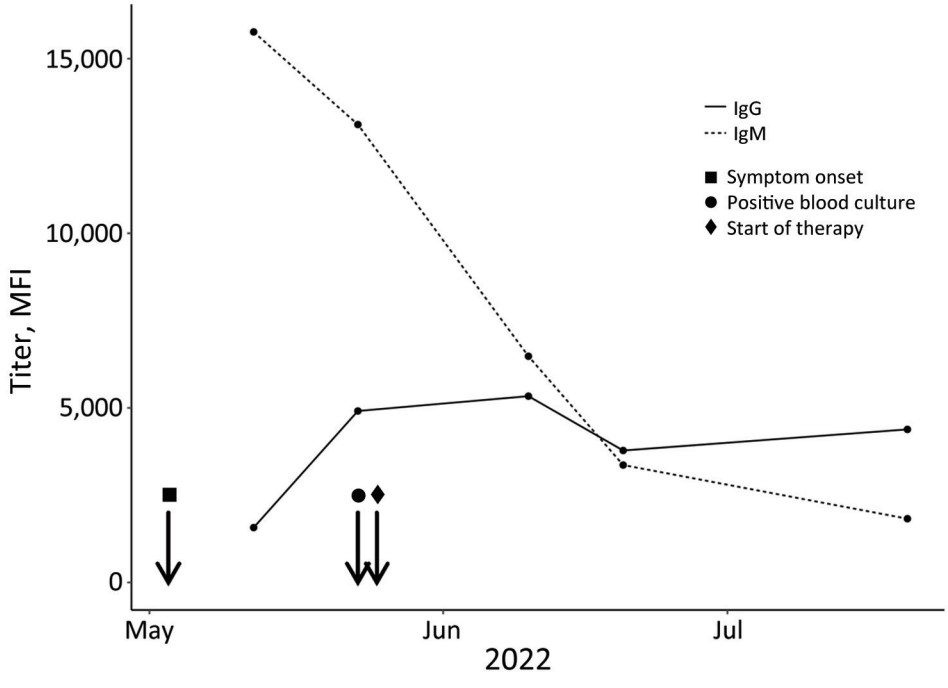

Figure 2. Antibody response to *Streptobacillus moniliformis* infection over time on 32-year-old female patient, Germany, 2022. The graph displays the dynamics of IgM (serum dilution 1:100) and IgG (serum dilution 1:250) levels in MFI values analyzed by *Streptobacillus* multiplex serologic tests (y-axis) and plotted against the time point of infection (x-axis). MFI, median fluorescence intensity.

After we identified the causative pathogen, we began an intravenous therapy with penicillin G (4 × 5 million IU) for 14 days. Because endocarditis was discovered late in the diagnostic process and no further complications arose, we continued monotherapy under frequent clinical and echocardiographic controls. After 14 days, we changed the therapy to oral amoxicillin (4 × 1 g) for another 4 weeks. Two weeks after the start of oral therapy, we no longer detected the aortic vegetation. Two weeks after therapy concluded, the patient reported well-being and no persistent symptoms.

We used a phylogenetic approach to group the microorganism from this study to closely related taxa (Appendix, https://wwwnc.cdc.gov/EID/article/30/3/23-0917-App1.pdf). The resulting tree confirmed the taxonomic position of the isolate from this study as a member of *S. moniliformis* bacteria.

In addition to microbiologic work-up, we analyzed serum samples from different time points for *S. moniliformis* bacteria–specific antibodies by using *Streptobacillus* multiplex serologic analysis. We found high IgM and IgG antibody levels in the patient's serum 9 days after symptom onset. IgM levels of subsequent measurements decreased, and IgG levels initially increased before declining approximately 3 weeks after the onset of symptoms (Figure 2).

Several aspects hamper the diagnosis of RBF, including unawareness of the disease among most clinicians, lack of reliable diagnostics, fastidious growth of the microorganism, susceptibility to most antibiotics used for empiric therapy (*3*), and unnoticed animal contact (*6*). Therefore, the incidence of RBF is unknown and difficult to estimate, especially because RBF is a nonnotifiable disease worldwide. Most of the published case reports do not properly identify the causative organism because they rely solely on 16S rRNA gene sequencing, which is insufficient for an accurate identification at species level (*6*).

In cases where direct detection methods, such as pathogen isolation or molecular testing, are not successful, serologic analysis could be a useful tool for clinical decision-making. High initial IgM and IgG levels of *S. moniliformis* bacteria–specific antibodies were measured in the patient by using *Streptobacillus* multiplex serologic analysis. However, because serologic tests for *S. moniliformis* bacteria are not commercially available nor readily accessible, the prevalence of RBF among humans is unknown. Further serologic studies could help to estimate the occurrence of RBF by shedding light on a largely unknown and underreported disease (*6*). Novel PCR tools could help to reduce the number of undetected infections and enable appropriate treatment.

This case report highlights the benefits of a One Health approach to healthcare in daily practice. Veterinary healthcare provided valuable information for clinicians regarding this rare disease and provided a serologic assay originally developed for the health monitoring of laboratory rodents and adapted for human application. Population-level serologic studies are needed to assess disease prevalence in high-risk groups. This case shows the possibility of species-specific RBF diagnosis in cases where direct diagnostic tools prove to be negative.

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Isolation of *Streptobacillus moniliformis* and IgM and IgG Immune Response in Patient with Endocarditis

Appendix

Methods used for phenotypic and molecular characterization of the *S. moniliformis* isolate

Phenotypic characterization

Bacterial isolation and physiologic properties

Bacterial isolates were identified using standard microbiological examination procedures. Briefly, venous aerobic and anaerobic blood cultures were incubated at 37°C, showing growth after 22 hours in the aerobic culture and after 4 days in the anaerobic culture. Isolates were subcultured on solid agar media (Columbia agar with 5% sheep blood [SBA; Oxoid, Wesel, Germany]. Agar plates were incubated for up to 48 h at 37°C using aerobic and microaerobic culture conditions. Phenotypic characterization of streptobacilli is known to yield only few weakly positive reactions (*1*), however, standard microbiological procedures included tests for hemolysis on SBA, catalase activity with 3% H_2O_2 on microscopic slides and for the presence of cytochrome oxidase with the BBL DrySlide® oxidase system (Becton–Dickinson, Heidelberg, Germany). Urease, hydrogen sulfide, indole, motility and oxidative and fermentative glucose assimilation were tested on Christensen agar, SIM and OF medium in slant agar tubes, respectively (all Merck, Darmstadt, Germany). Microscopic examinations of fixed smears were performed using Gram stain.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS)

For further identification attempts, the isolate was investigated by MALDI-TOF mass spectrometry. Mass spectrometry procedure has recently been described in detail (*2*,*3*). The commercial database used (DB 8,468; BrukerDaltonics) comprised 24 spectra each from 10 *S. moniliformis* strains. Reference spectra from well-characterized, quality-controlled strains of all

other *Streptobacillus*/*Pseudostreptobacillus* species and most other members of the *Leptotrichiaceae* were added to the database from previous studies (*2*,*4*). Identification was carried out with the commercial Bruker database, and with the extended database.

Molecular characterization

Whole genome sequencing and phylogenomic analysis

Whole genome sequencing (WGS) was carried out to get insight into a core genome based phylogeny and compare the patient's isolate with established field and type strain genomes from the same genus. The genome sequence of isolate 221009635 was determined by de-novo assembly with reads from Illumina technology. In brief, DNA was isolated from cells grown for 3 days at 37°C on SBA using a PureLink genomic DNA kit (Thermo Fisher). The library was prepared with DNA prep library preparation kit (Illumina) and sequenced on MiSeq instruments using v3 chemistry and a paired-end sequencing approach of 2×300 bp. Generated sequence data are deposited in the NCBI sequence read archieve (SRA) under the BioProject accession PRJNA946922. The genome assembly and quality assessment were carried out using the Aquamis pipeline (vers. 1.3.7), resulting in 179 contigs with 90x average coverage. Whole genome annotation was performed using prokka (vers. 1.14.6) (*5*).

A core genome phylogeny of strain 221009635 and 39 genomes of the genera *Streptobacillus/Pseudostreptobacillus* was calculated in EDGAR 3.0 based on MUSCLE alignment as previously described (*6*). This resulted in one multiple alignment of 481 core genes per genome (19,240 genes in total), with 169,393 aa residues per genome (6,775,720 in total). ANI values were computed as described by Goris et al. (*7*) and as implemented in JSpecies (*8*). The Neighbor-Joining algorithm was used for tree generation.

Species identity between the isolate and 27 other *S. moniliformis* genomes was confirmed by mean average nucleotide identity (ANI) values of >98.9% (data not shown), which is clearly above the >95%–96% proposed boundary for identical species (*7*). Based on an earlier published MLVA scheme for typing *S. moniliformis* (*9*) and by using WGS data, the isolate depicted the same genotype LHL11 like strains NCTC 11941 and Kun 3 (RIVM).

Methods used serologic investigation

Serology

The *S. moniliformis* serologic assay is part of the multiplex serology, which is used for routine health monitoring of laboratory rodent colonies at the DKFZ. The multiplex serology is based on an immunosorbent assay in combination with the fluorescent bead technology from Luminex Corp. (Austin, TX, USA). Membrane proteins of *S. moniliformis* Levaditi et al. type strain (ATCC 14647) were extracted and directly coupled to magnetic beads with an embedded fluorescent dye (MagPlex® Microspheres; Luminex Corp.) before incubation with serum (final dilution 1:100 for IgM and 1:250 for IgG analyses). The general set-up and protocol of the *Streptobacillus* multiplex serology is described by Schmidt et al. (manuscript in preparation). The Luminex analyzer BioPlex200 (BioRad Laboratories GmbH, Munich, Germany) was used to distinguish between the bead set and the bound antigen, and to quantify the amount of bound serum antibody by a secondary antibody (1:1000 dilution of biotinylated goat anti-human IgM or goat anti-human IgG, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) and a fluorescent reporter conjugate (1:500 Streptavidin-R-phycoerythrin). Final antigen-specific median fluorescence intensity (MFI) values were measured of at least 50 beads per bead set and serum sample.

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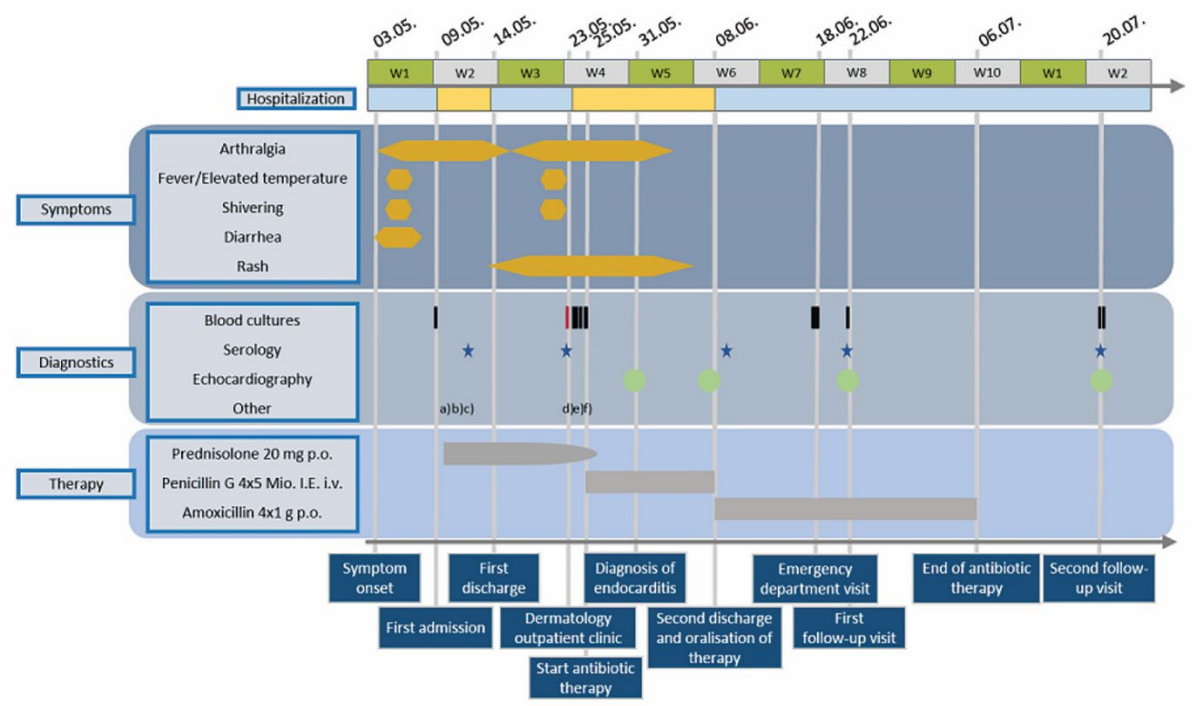
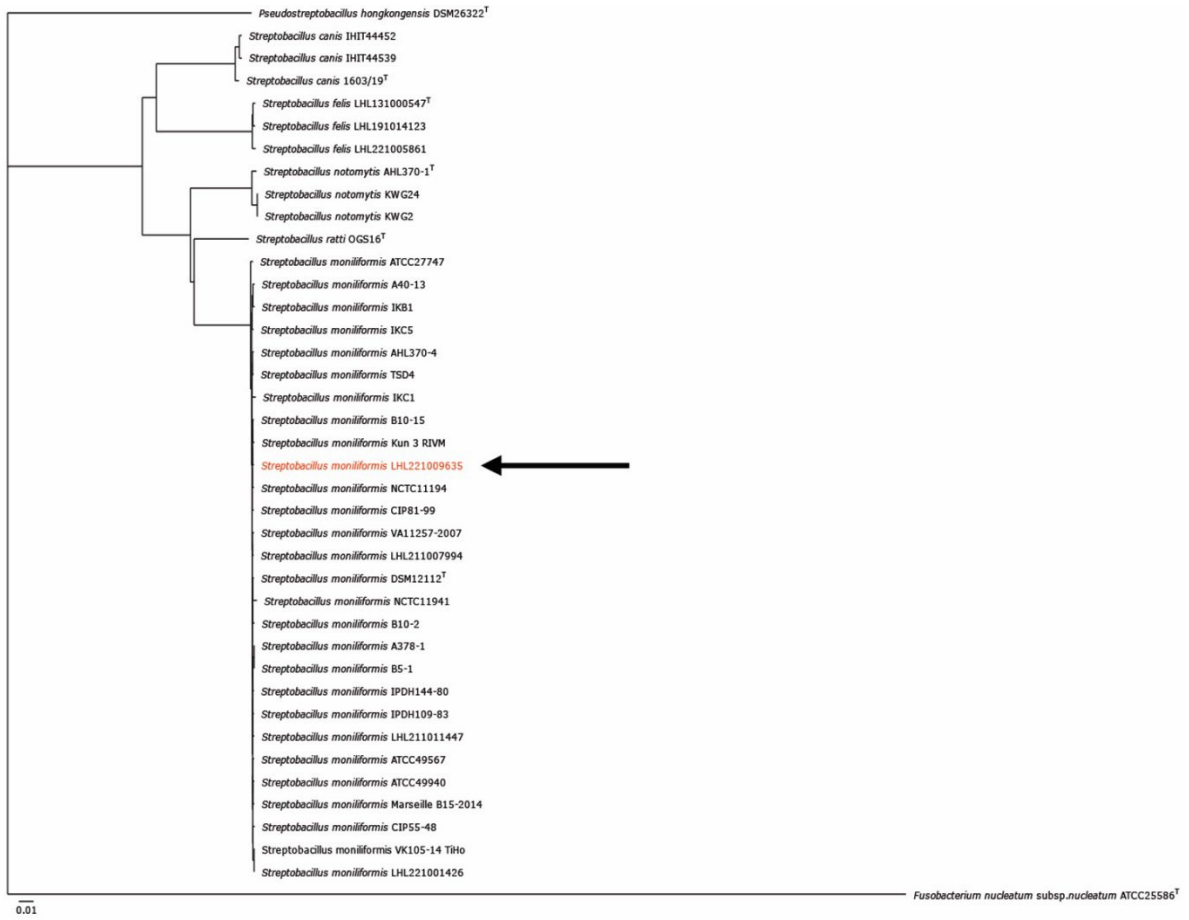
Appendix Table. Results of antimicrobial sensitivity testing using ETests (Liofilchem®)

Antibiotic agent	MIC (mg/l)
Penicillin G	0.25
Amoxicillin + Clavulanic acid	0.38
Ceftriaxon	0.023
Doxycyclin	≤0.016
Ciprofloxacin	0.19
Levofloxacin	0.19
Gentamicin	1
Rifampin	0.032

No susceptibility breakpoints exist for *Streptobacillus moniliformis*.



Appendix Figure 1. Time course of the *Streptobacillus moniliformis* infection from this study. The time course of the infection including performed diagnostic tests and results are displayed. The red bar in the diagnostics section represents the time point of the positive blood culture.



Appendix Figure 2. Core genome phylogenetic tree including the isolated *Streptobacillus moniliformis* strain. The core genome phylogenetic tree based on amino acid sequences depicts *Streptobacillus moniliformis* isolate 221009635 from this study within the genera *Streptobacillus/Pseudostreptobacillus*. Core genes of these genomes were computed in EDGAR 3.0 based on MUSCLE alignments and the Neighbor-Joining algorithm as implemented in the PHYLIP package. *Fusobacterium nucleatum* ssp. *nucleatum* is used as outgroup. Bar, 0.01 amino acid substitutions per site.