Supplementary Material for

Reoccurring bovine anthrax in Germany on the same pasture after 12 years

Peter Braun[1], Wolfgang Beyer[2], Matthias Hanczaruk[3], Julia M. Riehm[3], Markus Antwerpen[1], Christian Otterbein[4], Jacqueline Oesterheld[1] and Gregor Grass[1]*

[1]Bundeswehr Institute of Microbiology (IMB), Department of Bacteriology and Toxinology, Munich, Germany;
[2]Department of Livestock Infectiology and Environmental Hygiene, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany
[3]Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
[4]Local Veterinarian Unit, District Rosenheim, Germany
*Correspondence: gregorgrass@bundeswehr.org; Tel.: +49-992692-3981

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Abstract
The zoonotic disease anthrax caused by the endospore-forming bacterium *Bacillus anthracis* is very rare in Germany. In the state of Bavaria, the last case occurred in July of 2009 resulting in four dead cows. In August of 2021, the disease reemerged after heavy rains, killing one gestating cow. Notably, both outbreaks affected the same pasture, suggesting a close epidemiological connection. *B. anthracis* could be grown from blood culture and the presence of both virulence plasmids (pXO1 and pXO2) were confirmed by PCR. Also, recently developed diagnostic tools enabled rapid detection of *B. anthracis* cells and nucleic acids directly in clinical samples. The complete genome of the strain isolated from blood, designated BF-5, was DNA-sequenced and phylogenetically grouped within the B.Br.CNEVA clade that is typical for European *B. anthracis* strains. The genome was almost identical to BF-1, the isolate of 2009, separated only by three single nucleotide polymorphisms on the chromosome, one on plasmid pXO2 and three indel-regions. Further, *B. anthracis* DNA was detected by PCR from soil-samples taken from spots, where the cow had fallen onto the pasture. New tools based on phage receptor binding proteins enabled the microscopic detection and isolation of *B. anthracis* directly from soil-samples. These environmental isolates were genotyped and found to be SNP-identical to BF-1. Therefore, it seems that the BF-5 genotype is currently the prevalent one at the affected premises. The contaminated area was subsequently disinfected with formaldehyde.

Supplementary Table S1: Genome sequences accession numbers of newly sequenced and additional *B. anthracis* strains from publically available databases.

Strain designation	Country	Year	SNP-group	Accession number (chromosome)
Ames Ancestor	U.S.A.	1981	A.Br.Ames	NC_007530
BF-5	**Germany**	**2021**	**B.Br.CNEVA**	**SRR16572036**
BF-1	Germany	2009	B.Br.CNEVA	CP047131
Tyrol 6282	Austria	1979	B.Br.CNEVA	SRR10743038
Tyrol 3520	Austria	1980	B.Br.CNEVA	SRR10743039
Tyrol 3520	Austria	1980	B.Br.CNEVA	SRR10743039
Tyrol 4675	Austria	1988	B.Br.CNEVA	CP018903
A024	Slovakia	1972	B.Br.CNEVA	QAEL00000000
A016	Switzerland	*	B.Br.CNEVA	QAEM00000000
A046	Germany	*	B.Br.CNEVA	QAEN00000000
BA0188	Italy	2007	B.Br.CNEVA	SRR12435826
IMB 3011	**Italy**	**2005**	**B.Br.CNEVA**	**SRR16573065**
17OD930	Switzerland	2017	B.Br.CNEVA	SRR7100210
CNEVA-9066	France	1992	B.Br.CNEVA	NZ_AAEN01000000
ANSES 97-105	France	1997	B.Br.CNEVA	ERR1596585
ANSES 11-11_11	France	2011	B.Br.CNEVA	ERR1596595

bold: sequenced in this study; *information missing (Sahl et al., 2016**).

**Sahl JW, Pearson T, Okinaka R, Schupp JM, Gillece JD, Heaton H, et al. (2016) A *Bacillus anthracis* genome sequence from the Sverdlovsk 1979 autopsy specimens. MBio 7.

Supplementary Table S2: Primers used for SNP typing of additional isolates of *B. anthracis* BF-5 retrieved from contaminated soil at carcass site.

Primer Name (position)	Forward (5’-3’)	Reverse (5’-3’)
SNP 1	GCCACACTGGGACTGAGAC	GCCACCTACGTATTACCGCG
SNP 2	GAGGAAAGAGAGATGTTATTGTAGAAG	TACTCCTGGAACTCCTGAAGTAAC
SNP 3	TTGAGCCATTGTAAAAATCATCCCTTC	GTTTCTGTATCATAATATAAATAGTTGGGATG
SNP 4	ATTTCTTCTTTCAGGTACATAATAAAAGCAG	CTAAAGGAGTTGACGTATTAATGGAAG

Supplementary Table S3: Ct values of 16S rRNA SNP (RT)-PCR of total nucleic acids from blood samples.

Sample	Ct value 16S rRNA SNP-PCR	Ct value 16S rRNA SNP RT-PCR
Inactivated blood 1:10	24.9	not done
Inactivated blood 1:100	28.1	not done
Inactivated blood 1:1000	31.7	not done
Nucleic acid extraction from blood 1:10	13.9	9.7
Nucleic acid extraction from blood 1:100	18.1	12.8
Nucleic acid extraction from blood 1:1000	21.5	17.8
H_2O (negative control)	-	-

* total nucleic acids were extracted from inactivated cow blood samples.