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Prosthetic Valve Endocarditis with *Bartonella washoensis* in a Human European Patient and Its Detection in Red Squirrels (*Sciurus vulgaris*)

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ABSTRACT Members of the genus *Bartonella* are fastidious Gram-negative facultative intracellular bacteria that are typically transmitted by arthropod vectors. Several *Bartonella* spp. have been found to cause culture-negative endocarditis in humans. Here, we report the case of a 75-year-old German woman with prosthetic valve endocarditis due to *Bartonella washoensis*. The infecting agent was characterized by sequencing of six housekeeping genes (16S rRNA, *ftsZ*, *gltA*, *groEL*, *ribC*, and *rpoB*), applying a multilocus sequence typing (MLST) approach. The 5,097 bp of the concatenated housekeeping gene sequence from the patient were 99.0% identical to a sequence from a *B. washoensis* strain isolated from a red squirrel (*Sciurus vulgaris orientis*) from China. A total of 39% (24/62) of red squirrel (*S. vulgaris*) samples from the Netherlands were positive for the *B. washoensis gltA* gene variant detected in the patient. This suggests that the red squirrel is the reservoir host for human infection in Europe.

KEYWORDS *Bartonella washoensis*, Europe, *Sciurus vulgaris*, endocarditis, human, multilocus sequence typing (MLST), red squirrel, reservoir

Members of the genus *Bartonella* are fastidious Gram-negative facultative intracellular bacteria that are typically transmitted by arthropod vectors (1). *Bartonella* spp. are distributed worldwide and have been found to cause clinically overt disease, mainly in humans and dogs (2). Further, they have been detected in a large array of potential reservoir hosts, in which they establish a persistent intraerythrocytic bacteremia (3). The most relevant species for human health are *Bartonella bacilliformis*, *B. quintana*, and *B. henselae*. However, in recent years other *Bartonella* spp. were recognized as human pathogenic as well, although they have been reported to affect humans only infrequently. The most prominent disease that they cause is culture-negative endocarditis (1).

Only two human infections with *B. washoensis* have been reported so far. *B. washoensis* was isolated in North America from the blood of two patients with myocarditis (4) and meningitis (5), respectively. Further, it was cultured from the blood of a Californian dog with endocarditis (6). Squirrels were suggested to be the reservoir hosts for human infection, because the 16S rRNA, *groEL*, and *gltA* sequences of *B. washoensis* strain Sb944nv cultured from a Californian ground squirrel (*Spermophilus beecheyi*) were 100% identical to those from the patient with myocarditis mentioned above (4). In North America, one of the arthropod vectors that might transmit *B.*

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washoensis to humans is the *S. beecheyi*-parasitizing flea *Oropsylla montana*, because *gltA* sequences from fleas of this species were 100% identical to those from human infection (5).

Here, we report the case of a 75-year-old German woman with prosthetic valve endocarditis due to *B. washoensis*, the molecular characterization of the infectious agent using a multilocus sequence typing (MLST) approach, and its detection in European red squirrels (*Sciurus vulgaris*).

MATERIALS AND METHODS

Laboratory tests. Mitral valve tissue was plated on Columbia blood agar and chocolate agar and incubated with 5% CO_2 for 4 weeks. DNA was extracted using a QIAamp DNA minikit (Qiagen, Hilden, Germany). A broad-range PCR targeting the 16S rRNA gene was performed as described previously (7). Blood cultures were held for 4 weeks, applying a Bactec blood culture system (BD Diagnostics, Heidelberg, Germany). Mitral valve tissue and serum were sent for histopathological analysis and quantification of specific antibodies by the use of an immunofluorescence test detecting anti-*B. henselae* and anti-*B. quintana* IgG and IgM.

PCR and sequencing. The infecting agent was further characterized by amplification and bidirectional sequencing of six housekeeping genes (16S rRNA, *ftsZ*, *gltA*, *groEL*, *ribC*, and *rpoB*), applying a MLST approach described previously (8). For amplification of a larger part of the 16S rRNA gene, primers Bw16Sgesf (GCCAAATCGAATTTTCAATATG) and Bw16Sgesr (TCCAATTCTTGATCATCCTTAG) were newly designed. DNA (2 μl) was used as a template in a 50-μl reaction mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, a 0.4 μM concentration of each primer, and 0.2 μl (1 U) of *Taq* DNA polymerase (Invitrogen, Karlsruhe, Germany). The PCR was performed by the use of a model 2720 GeneAmp thermal cycler (Applied Biosystems, Darmstadt, Germany) under the following conditions: an initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, extension at 72°C for 1 min 30 s, and a final extension at 72°C for 10 min. For bidirectional sequencing, primers Bw16Sseqf (TTAGTTGCCAGCATTCAGTTG) and Bw16Sseqr (GCTGCTGGCACGAAGTTAG) were used additionally. Because the BhCS781.p and BhCS1137.n primers described previously by Norman et al. (9) did not yield any product due to primer mismatches, new primers BwgltAgesf (AAGGATCTGAAAGGAATATCTA) and BwgltArmod (ACGCGCTGCATAGCCTGTAT) had to be developed for the amplification of *gltA*, taking into account the whole-genome shotgun sequences of *B. washoensis* available at GenBank (accession numbers UAQI01000040, NZ_JH725101, and NZ_JH725022). The PCR conditions were the same as described above, with the exception of an annealing step at 50°C for 30 s. For bidirectional sequencing, primers BwgltAf (AATCAATCCAGTGCTTA CTCG) and BwgltAr (CTG CATAGCCTGTATAGAGTT) were used additionally.

Phylogenetic analysis. *B. washoensis* strain human_1487_18 reported here was compared to 20 *B. washoensis* isolates collected from squirrels in North America and China, described earlier (8). Phylogenetic analysis was performed using the program MEGA X (10). The concatenated sequences of six housekeeping genes were aligned by ClustalW, applying the IUB matrix. Tree construction was achieved by the neighbor-joining method using the Jukes-Cantor model with the complete-deletion option. Bootstrap analysis was conducted with 1,000 replicates. Pairwise distances between the concatenated housekeeping gene sequences were computed by using the Jukes-Cantor matrix and applying the complete deletion option.

Squirrel samples. One of the tasks of the Dutch Wildlife Health Center in Utrecht is pathogen surveillance in wildlife. Therefore, the public was asked to report squirrels found dead to the center. Sixty-two red squirrels (*S. vulgaris*) that were submitted between 2015 and 2017 were investigated for the *B. washoensis* strain described here. Collection site, year of collection, age status, and sex of the squirrels are given in Table 1. The collection sites of the squirrels are illustrated in Fig. 1. DNA was isolated from the spleen using a DNeasy blood and tissue kit (Qiagen, Hilden, Germany). Because primers BwgltAgesf and BwgltArmod yielded unspecific products, primers BwgltAf and BwgltAr were applied for amplification and bidirectional sequencing under the conditions described above, with the exception of an extension at 72°C for 1 min. For phylogenetic analysis, the obtained *gltA* sequences were compared to sequences from the three human strains known so far and to sequences from squirrel strains collected in North America and China under the conditions described above.

Data availability. The sequences detected here were submitted to GenBank and are available under the accession numbers MN220719 and MN229488 to MN229502. A MLST database for *B. washoensis* was established. It is hosted on PubMLST (https://pubmlst.org/databases/) and contains the allelic profiles of the human strain 1487_18 and of the 20 *B. washoensis* isolates from squirrels described earlier (8).

RESULTS

Case presentation. In 2013, a female German patient received a biological mitral valve prosthesis because of mitral insufficiency. In 2018, at the age of 75, she presented with chronic cardiac decompensation because of mitral valve stenosis. No clinical or echocardiographic signs of endocarditis were evident. The indication for mitral valve re-replacement was made. Intraoperatively, cauliflower-like plaques were observed on the mitral valve leaflets. Conventional culture from prosthetic valve tissue and blood

TABLE 1 Collection site, year of collection, estimated age, and sex of the 62 red squirrels (*Sciurus vulgaris*) from the Netherlands that were investigated for *Bartonella washoensis* infection by PCR analysis of their spleen samples[a]

Animal no.	Collection site	Province	Yr collected	Age status	Sex	PCR result
1	Heerde	Gelderland	2015	Juvenile	F	−
2	Epe	Gelderland	2015	Adult	M	−
3	Venlo	Limburg	2015	Adult	M	−
4	Amersfoort	Utrecht	2015	Adult	F	−
5	Amersfoort	Utrecht	2015	Adult	M	−
6	Berg en Terblijt	Limburg	2015	Adult	F	−
7	Boekelo	Overijssel	2015	Adult	F	−
8	**Vaassen**	**Gelderland**	**2015**	**Adult**	**M**	**+**
9	**Heeze**	**North Brabant**	**2015**	**Adult**	**M**	**+**
10	**Best**	**North Brabant**	**2015**	**Adult**	**M**	**+**
11	Vorden	Gelderland	2015	Adult	M	−
12	Diever	Drenthe	2015	Juvenile	M	−
13	Diever	Drenthe	2015	Juvenile	F	−
14	Wapse	Drenthe	2015	Adult	M	−
15	Bennekom	Gelderland	2015	Juvenile	M	−
16	Hoeven	North Brabant	2015	Adult	F	−
17	Hattem	Gelderland	2015	Juvenile	M	−
18	**Utrechtse Heuvelrug**	**Utrecht**	**2015**	**Juvenile**	**M**	**+**
19	Hezingen	Overijssel	2015	Juvenile	F	−
20	Steyl	Limburg	2015	Juvenile	F	−
21	Lunteren	Gelderland	2015	Juvenile	M	−
22	**Borne**	**Overijssel**	**2015**	**Adult**	**M**	**+**
23	Boekel	North Brabant	2015	Adult	F	−
24	Apeldoorn	Gelderland	2015	Adult	M	−
25	Apeldoorn	Gelderland	2015	Adult	M	−
26	Markelo	Overijssel	2015	Juvenile	F	−
27	**Utrecht**	**Utrecht**	**2015**	**Adult**	**M**	**+**
28	**Uden**	**North Brabant**	**2015**	**Adult**	**F**	**+**
29	Oldebroek	Gelderland	2015	Adult	M	−
30	Gieten	Drenthe	2015	Adult	M	−
31	**Bergen op Zoom**	**North Brabant**	**2015**	**Adult**	**F**	**+**
32	Geldrop	North Brabant	2015	Juvenile	M	−
33	**Joppe**	**Gelderland**	**2016**	**Adult**	**F**	**+**
34	**Amersfoort**	**Utrecht**	**2016**	**Adult**	**M**	**+**
35	Barneveld	Gelderland	2016	Adult	F	−
36	Ulft	Gelderland	2016	Adult	F	−
37	**Wilhelminaoord**	**Drenthe**	**2016**	**Adult**	**M**	**+**
38	**Breda**	**North Brabant**	**2016**	**Adult**	**F**	**+**
39	Norg	Drenthe	2016	Adult	M	−
40	**Wilhelminaoord**	**Drenthe**	**2016**	**Adult**	**F**	**+**
41	Wilhelminaoord	Drenthe	2016	Adult	F	−
42	**Neede**	**Gelderland**	**2016**	**Adult**	**M**	**+**
43	**Noordlaren**	**Groningen**	**2016**	**Adult**	**F**	**+**
44	**Bosschenhoofd**	**North Brabant**	**2016**	**Juvenile**	**M**	**+**
45	**Lunteren**	**Gelderland**	**2016**	**Adult**	**M**	**+**
46	Putte	North Brabant	2016	Adult	F	−
47	Hardenberg	Overijssel	2016	Adult	F	−
48	**Winterswijk**	**Gelderland**	**2016**	**Juvenile**	**F**	**+**
49	Hengelo	Overijssel	2016	Adult	F	−
50	**Roermond**	**Limburg**	**2017**	**Adult**	**F**	**+**
51	**Epe**	**Gelderland**	**2017**	**Adult**	**F**	**+**
52	**Huizen**	**Noord-Holland**	**2017**	**Adult**	**M**	**+**
53	Wijster	Drenthe	2017	Nd	Nd	−
54	Kaatsheuvel	North Brabant	2017	Juvenile	M	−
55	Rucphen	North Brabant	2017	Adult	F	−
56	Loon	Drenthe	2017	Adult	M	−
57	**Zeist**	**Utrecht**	**2017**	**Juvenile**	**F**	**+**
58	Gasselte	Drenthe	2017	Juvenile	M	−
59	Lunteren	Gelderland	2017	Juvenile	M	−
60	Ermelo	Gelderland	2017	Adult	M	−
61	**Havelte**	**Drenthe**	**2017**	**Juvenile**	**M**	**+**
62	**Epe**	**Gelderland**	**2017**	**Adult**	**F**	**+**

[a]F, female; M, male; Nd, not determined. Data representing animals with positive results are highlighted in bold.

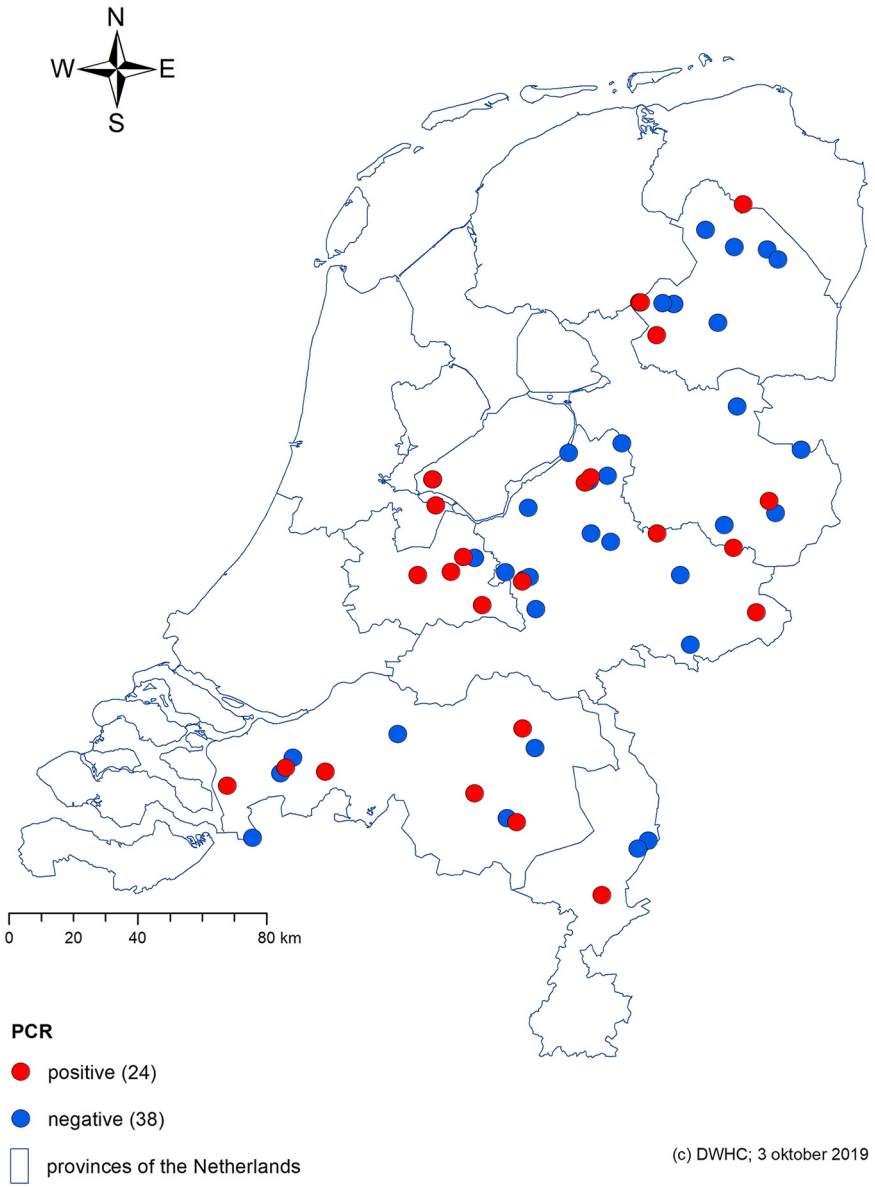

FIG 1 Geographical map of the Netherlands illustrating the collection sites of the 62 red squirrels (*Sciurus vulgaris*) that were investigated for *Bartonella washoensis* infection by PCR.

cultures did not yield any growth. However, the result of broad-range PCR analysis of the prosthetic valve tissue was positive and the respective 16S rRNA gene sequence was 99.8% (410/411 bp) identical to that of *B. washoensis* strain 08S-0475 (GenBank accession number FJ719017) from the patient with meningitis mentioned above (5). The histopathological analysis was consistent with active endocarditis. IgG antibody titers of 1:2048 against *B. henselae* and of 1:256 against *B. quintana* were found, whereas anti-*Bartonella* IgM antibodies were not detected. The patient denied contact with animals and their ectoparasites. Treatment with doxycycline for 6 weeks and gentamicin for 2 weeks was recommended. Unfortunately, the patient died of unspecified reasons 4 months later.

Molecular characterization of the patient strain. The 16S rRNA gene sequence from the patient was amplified with specific primers (8) and was found to be 99.9% (1,347/1,349 bp) identical to that from a *B. washoensis* strain from a red squirrel (*S. vulgaris orientis*) from China (GenBank accession number AB519066). When the longer

16S rRNA gene sequence generated with primers Bw16Sgesf and Bw16Sgesr was compared to GenBank, an identity of 99.5% (1,474/1,481 bp without one gap) to an uncharacterized *Bartonella* sp. from a Daurian ground squirrel (*Spermophilus dauricus*) (GenBank accession number DQ641912) was detected.

The *gltA* sequence was found to be 99.7% identical to a sequence from a *B. washoensis* strain from a red squirrel (*S. vulgaris orientis*) in China (335/336 bp; GenBank accession number AB444974) and to a sequence from a strain described as *Bartonella* sp. from a red squirrel (*S. vulgaris*) in the United Kingdom (329/330 bp, GenBank accession number AF449760). Comparison of the longer *gltA* sequence amplified with primers BwgltAgesf and BwgltArmod to GenBank showed that no significant match was obtained (90.1% identity to *B. henselae*).

An observation similar to that received with the shorter *gltA* sequence was made for the *groEL* gene, showing identity of 99.6% (1,180/1,185 bp) to the strain from China (GenBank accession number AB519097) and identity of 98.6% (1,189/1,206 bp) to the strain from the United Kingdom (GenBank accession number AF449762).

ftsZ, *ribC*, and *rpoB* sequences were available at GenBank only for the Chinese strain. Results of comparisons showed that 785/788 bp (99.6%) of the *ftsZ* gene (GenBank accession number AB519080), 586/618 bp (94.8%) of the *ribC* gene (GenBank accession number AB519114), and 818/825 bp (99.2%) of the *rpoB* gene (GenBank accession number AB519129) of the *B. washoensis* strain described here were identical to the corresponding sequences from the red squirrel strain (*S. vulgaris orientis*) from China.

The patient strain described here was also compared to the two human *B. washoensis* isolates from North America (4, 5). Their 16S rRNA gene sequences were 99.6% identical to those from the European strain as follows: 1,406/1,412 bp (without one gap) of human isolate NVH1 (GenBank accession number AF070463) and 1,318/1,323 (without one gap) of human isolate 08S-0475 (GenBank accession number FJ719017). A higher level of diversity was evident in the *gltA* and *groEL* sequences. For *gltA*, identity of 94.4% (319/338 bp) was observed for isolate NVH1 (GenBank accession number AF050108) and identity of 92.6% (311/316 bp) for isolate 08S-0475 (GenBank accession number FJ719016). A similar observation was made for *groEL*, with identity to the European patient strain of 93.6% (1,264/1,350 bp without six gaps) for isolate NVH1 (GenBank accession number AF071193) and identity of 95.2% (511/537 bp) for isolate 08S-0475 (GenBank accession number FJ695137).

Phylogenetic analysis. The six housekeeping genes (16S rRNA, *ftsZ*, *gltA*, *groEL*, *ribC*, and *rpoB*) were concatenated in frame (5,097 bp) and compared to those from 20 *B. washoensis* strains from squirrels from North America and China reported earlier (8), and the results showed that the strain described here formed a separate cluster with red squirrel strain ER14-3 from China mentioned above (Fig. 2).

The 5,097 bp of the concatenated housekeeping gene sequence from the patient were 99.0% identical to the red squirrel (*S. vulgaris orientis*) strain from China. The 1,537 bp of the concatenated *gltA* and *groEL* sequences of red squirrel strain SC12uk from the United Kingdom mentioned above were compared to the *B. washoensis* strain described here, and the identity was found to be 98.8%.

The analyses showed that 2,185 bp of the concatenated 16S, *gltA*, and *groEL* sequences of the two human strains from North America, NVH1 (4) and 08S-0475 (5), were 99.6% identical to each other. Compared to European *B. washoensis* strain human_1487_18 described here, the identity was 97.5% for both North American isolates.

Squirrel samples. A total of 39% (24/62) red squirrel (*S. vulgaris*) spleen samples were positive for *B. washoensis* in experiments in which primers BwgltAf and BwgltAr, which amplify 584 bp (without primers) of the *gltA* gene, were used. Notably, primers BhCS781.p (four mismatches, one of them near the 3′ end) and BhCS1137.n (one mismatch), previously described by Norman et al. (9), did not yield any product because of mispriming. Ten positive PCR products were randomly chosen and bidirectionally

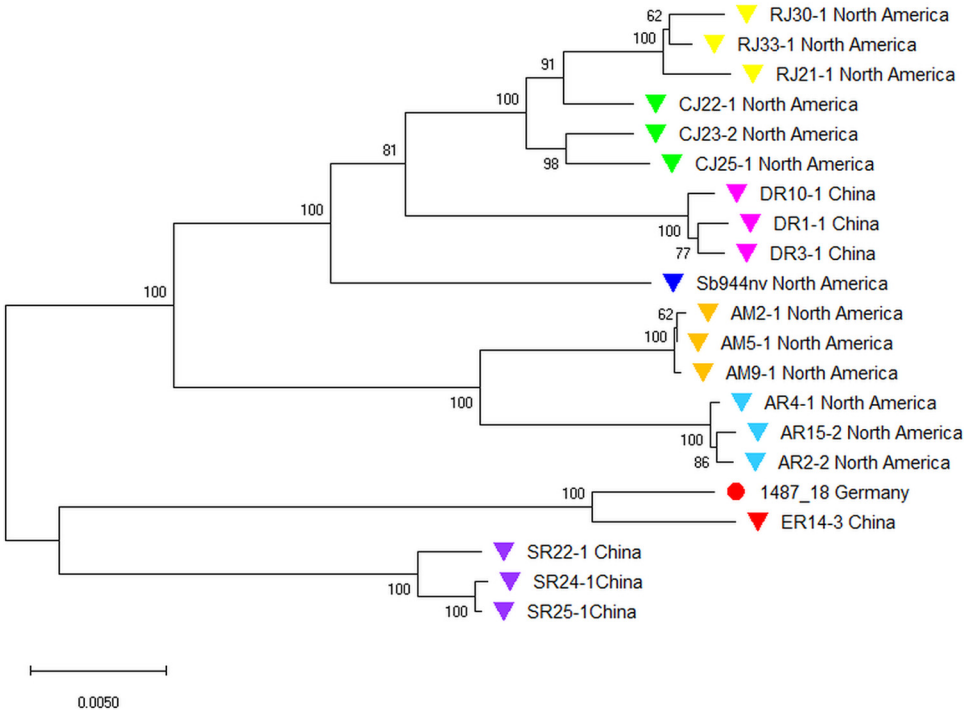

FIG 2 Neighbor-joining phylogenetic tree calculated from the concatenated housekeeping gene sequences (16S rRNA, *ftsZ*, *gltA*, *groEL*, *ribC*, and *rpoB*). The scale bar indicates the number of nucleotide substitutions per site. Bootstrap values are shown next to the branches. The final data set contained 5,094 positions. Yellow inverted triangle, *Spermophilus richardsonii*; green inverted triangle, *S. columbianus*; fuchsia inverted triangle, *S. dauricus*; dark blue inverted triangle, *S. beecheyi*; ocher inverted triangle, *Glaucomys volans*; light blue inverted triangle, *Tamiasciurus hudsonicus*; red circle, *Homo sapiens*; red inverted triangle, *Sciurus vulgaris orientis*; purple inverted triangle, *Tamias sibiricus*.

sequenced. The respective sequences were 100% identical to each other and to the patient's sequence.

For phylogenetic analysis, this unique European *gltA* sequence was compared to *B. washoensis gltA* sequences from humans and squirrels from North America and China (Fig. 3). Strains from red squirrels (*S. vulgaris*) and the European patient were separated (with high bootstrap support of 100%) from those from Californian ground squirrels (*S.*

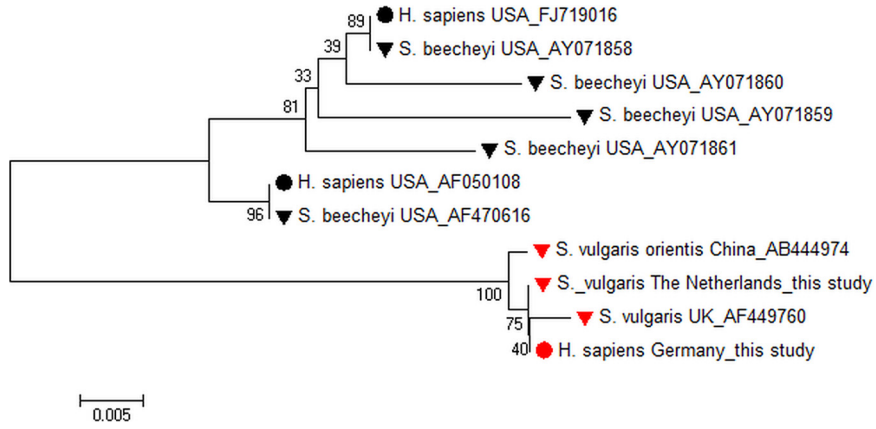

FIG 3 Neighbor-joining phylogenetic tree calculated from *gltA* sequences obtained during this study and from GenBank. The GenBank accession numbers are given after the underscore. The scale bar indicates the number of nucleotide substitutions per site. Bootstrap values are shown next to the branches. The final data set contained 320 positions. red circle, *Homo sapiens* (Europe); red inverted triangle, *Sciurus vulgaris* (*orientis*); black circle, *Homo sapiens* (North America); black inverted triangle, *Spermophilus beecheyi*.

beecheyi) and the two North American patients. However, the significance of this conclusion is limited, because the final data set contained only 320 bp due to the different lengths of the *gltA* sequences available.

DISCUSSION

We report here what was, to our knowledge, the first human infection with *B. washoensis* in Europe, as the two other known cases occurred in North America (4, 5). Further, this report represents the first recognition of *B. washoensis* as a causative agent of human endocarditis, although it had been isolated earlier from a Californian dog with endocarditis (6). Interestingly, humans and dogs have been described to suffer from similar disease manifestations due to *Bartonella* species infections (2), including endocarditis (11). Apart from the common species *B. quintana* and *B. henselae*, several other *Bartonella* spp. have been shown to cause culture-negative endocarditis (1). Thus, this seems to be a general feature of the genus *Bartonella*.

In a small case series of 48 patients, preexisting valvular heart disease was associated with *B. henselae* but not with *B. quintana* endocarditis (12). However, as valvular heart disease is a major risk factor for endocarditis in general, the mitral valve stenosis of her prosthetic valve probably predisposed our patient to such disease. Typically, patients with *Bartonella* endocarditis present with nonspecific symptoms such as fever, fatigue, and weight loss (1), but such was not the case in our patient. Echocardiographic evidence of vegetation has been described previously to be not as easily detectable in *Bartonella* endocarditis as in other forms of endocarditis (1). This was also true for the patient presented here, as no echocardiographic signs of endocarditis were evident preoperatively. However, intraoperatively, cauliflower-like plaques were observed on the mitral valve leaflets and the histopathological analysis was consistent with active endocarditis.

A *Bartonella* IgG titer of $\geq$1:800 is regarded as a cutoff value associated with a high positive predictive value for endocarditis (1). Broad serological cross-reactivity between members of the genus *Bartonella* has been observed previously (1). Thus, in our patient, *B. henselae* and *B. quintana* were used as surrogate antigens, yielding an IgG antibody titer of 1:2,048 for *B. henselae* in the immunofluorescence test, which was substantially above the cutoff level of 1:800. The immunofluorescence test is considered to be not species specific and shows considerable cross-reactivity between *Bartonella* spp. (1), as further supported by our findings. Although there is no clinical experience in the management of *B. washoensis* endocarditis, treatment for *Bartonella* species endocarditis according to current guidelines was recommended (13).

Squirrels were suggested to be the reservoir for human infection, because the 16S rRNA, *groEL*, and *gltA* sequences of *B. washoensis* strain Sb944nv cultured from a Californian ground squirrel (*S. beecheyi*) were 100% identical to those from a human patient (4). The *B. washoensis* human_1487_18 strain described here showed the highest nucleotide identities with red squirrel strain ER14-3 from *S. vulgaris orientis* from China and with red squirrel strain SC12uk from *S. vulgaris* from the United Kingdom. The red squirrel strain from the United Kingdom was described as *Bartonella* sp. because it did not group with known *Bartonella* spp. in the phylogenetic analysis at the time of publication (14). However, we compared it to GenBank strains and found that its *gltA* sequence was 99.4% (329/331 bp) identical to that of red squirrel strain ER14-3 (GenBank accession number AB444974) from China and 94.0% (311/331 bp) identical to a *B. washoensis* strain from *S. beecheyi* from North America (GenBank accession number AF470616). A similar observation was made for its *groEL* sequence, which showed identity of 98.4% (1,166/1,185 bp) to red squirrel strain ER14-3 (GenBank accession number AB519097) and of 96.1% (1,139/1,185 bp) to a *B. washoensis* strain from a Siberian chipmunk (*Tamias sibiricus*) (GenBank accession number AB519090). Therefore, red squirrel strain SC12uk might represent *B. washoensis* as well.

On the other hand, red squirrel strain ER14-3 and our human 1487_18 strain formed a separated cluster in the phylogenetic analysis (Fig. 2). Thus, these strains might belong to a subspecies that has yet to be defined. A similar observation was made for

B. washoensis isolates from black-tailed prairie dogs (*Cynomys ludovicianus*) that were previously proposed to form the subspecies "*Candidatus* B. washoensis subsp. cynomysii" (15). The name of this subspecies was later corrected to "*Candidatus* B. washoensis subsp. cynomyis" (16).

To prove that the *B. washoensis* strain described here was present in the European red squirrel (*S. vulgaris*) population, we investigated 62 animals from the Netherlands. A relevant proportion of 39% (24/62) was found to be infected, and their *gltA* sequences were 100% identical to our patient strain. Thus, red squirrels might serve as reservoir hosts for human infection. Note that the widely used primers for screening of *Bartonella* infection described by Norman et al. (9) did not amplify the *B. washoensis gltA* variant we described here. An infection rate of 69% (97/140) with *B. washoensis* was reported for the Californian ground squirrel (*S. beecheyi*) (17), which is somewhat higher than the infection rate of 39% that we detected in red squirrels. Carnivores such as the Japanese marten (*Martes melampus*) might not be the reservoir for human infection with the *B. washoensis* variant that we describe here, because its concatenated housekeeping gene sequence (16S rRNA, *ftsZ*, *gltA*, *groEL*, *ribC*, and *rpoB*) did not cluster with red squirrel strain ER14-3 (18).

The *gltA* sequences from squirrels and humans available at GenBank were compared to the unique *gltA* sequence found here, and the results showed that the strains from humans and squirrels from North America as well as from Europe and Asia clustered together (Fig. 2). Thus, instead of a separate subspecies, strain human_1487_18 could represent a Eurasian variant of *B. washoensis* pathogenic for humans.

In North America, one of the arthropod vectors that might transmit *B. washoensis* to humans is the *S. beecheyi*-parasitizing flea *O. montana* because *gltA* sequences from fleas of this species were 100% identical to those from human infection (5). However, flea transmission of *B. washoensis* has not been proven experimentally (3). In Europe, red squirrels (*S. vulgaris*) are mainly parasitized by the flea *Ceratophyllus sciurorum*. It has to be shown in the future which *B. washoensis* variants might be present in *C. sciurorum* to gain evidence about its vector competence for *B. washoensis*.

At the least, patients with *B. henselae* endocarditis reported significantly more frequent exposure to cats or cat fleas than the controls (12). Our patient denied contact with animals and their ectoparasites. This, however, does not exclude the possibility that she was infected via transmission by fleas, because it is a common finding in vector-borne diseases in general that the exposure to the vector is not recognized. For example, only 60% of patients with erythema migrans due to *Borrelia burgdorferi* infection recalled the tick bite (19).

In conclusion, we present the first human case of *B. washoensis* endocarditis. Further studies are needed to show whether other reservoir hosts apart from red squirrels (*S. vulgaris*) might harbor human-pathogenic *B. washoensis* variants and whether fleas parasitizing red squirrels might be involved in their transmission.

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