Bartonella schoenbuchii sp. nov., isolated from the blood of wild roe deer

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The genus Bartonella comprises two human-specific pathogens and a growing number of zoonotic or animal-specific species. Domesticated as well as wild mammals can serve as reservoir hosts for the zoonotic agents and transmission to humans may occur by blood sucking arthropods or by direct blood to blood contact. Humans may come into intimate contact with free-ranging mammals during hunting, especially during evisceration with bare hands, when accidental blood to blood contact frequently occurs. The objective of this work was to determine the presence and the polymorphism of Bartonella strains in wild roe deer (Capreolus capreolus) as the most widely spread game in Western Europe. We report the isolation of four Bartonella strains from the blood of five roe deer. These strains carry polar flagella similar to Bartonella bacilliformis and Bartonella clarridgeiae. Based on their phenotypic and genotypic characteristics, three of the four roe deer isolates were different and they were all distinct from previously described Bartonella species. They can be distinguished from each other and from other Bartonella species by their protein profile, ERIC-PCR pattern, 16S rRNA and citrate synthase (gltA) gene sequences, as well as by whole DNA–DNA hybridization. In spite of their considerable heterogeneity, all four strains fulfill the criteria for belonging to a single new species. The name Bartonella schoenbuchii is proposed for this new species. The type strain R1T of Bartonella schoenbuchii has been deposited in the National Collection of Type Cultures as NCTC 13165T and the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 13525T.

INTRODUCTION

The genus Bartonella comprises small, curved, often piliated or flagellated bacteria, which have been cultured in recent years on axenic media from the blood of many mammalian hosts (Dehio & Sander, 1999). Together with the reclassification of bacteria formerly belonging to the genera Rochalimaea and Grahameilla (Brenner et al., 1993; Birtles et al., 1995) this has resulted in a dramatic expansion of the genus Bartonella from one described species in 1993 to currently 15 species, among which at least seven have been associated with human disease (Dehio & Sander, 1999). A haemotropic life style together with transmission by blood-sucking arthropods appears to provide a unique parasitic strategy for all Bartonella spp. However, each species seems to be highly adapted to one or a few mammalian hosts, causing a haemotropic infection characterized by the ability to parasitize erythrocytes intracellularly. Two species, Bartonella bacilliformis and Bartonella quintana, are adapted to humans as reservoir host causing oroya fever/verruga peruana and trench fever, respectively (Dehio & Sander, 1999). In contrast, incidental infection of humans as non-reservoir hosts does not appear to lead to erythrocyte invasion but may cause various clinical manifestations (Dehio & Sander, 1999). Transmission to a non-reservoir host can either occur directly via
blood to blood contact or indirectly via haematopha-
gous arthropods such as fleas or lice. For public health
management, it is important to evaluate the presence of *Bartonella* species as a potential cause of zoonosis
in those animals to which humans are prominently
exposed. Cat-associated *Bartonella henselae* and
*Bartonella clarridgeiae* (Garfield et al., 1997; Heller et
al., 1997; Kordick et al., 1997), *Bartonella elizabethae*
naturally infecting rats (Daly et al., 1993; Ellis et al.,
1999), *Bartonella grahamii* (Kerkhoff et al., 1999) and
*Bartonella vinsonii* subsp. *berkhoffii* from a dog reservoir (Roux et al.,
2000), have all been associated with human infection
and therefore represent zoonotic pathogens.

In this study we have investigated the presence of
*Bartonella* in wild roe deer (*Capreolus capreolus*). These
animals represent the most widely spread game in
Western Europe that is hunted in large numbers (in
Germany more than 1000000 animals are shot per
year). By typical hunting practice, hunters and forestry
officials (> 300000 individuals in Germany) are ex-
dosed to direct contact with animal blood, especially
during evisceration with bare hands. Accidental injury
may bear the risk of direct blood to blood contact and
result in infection with *Bartonella* spp. Moreover, a
recent study detected *Bartonella* DNA in more than
70% of the ticks collected from roe deer (Schouls et al.,
1999), suggesting that ticks may serve as an arthropod
vector for transmission of bartonellae. Here we report
the isolation of a new *Bartonella* species from the
blood of wild roe deer. The prevalence of this species in
roe deer of south-western Germany seems to be high
and strains belonging to this new species appear to be
highly diverse.

**METHODS**

**Culture of Bartonella type and reference strains.** *Bartonella*
ssp. were grown on Columbia agar base with 5%
defibrinated sheep blood. Plates were incubated at 37 °C in
a moist atmosphere for up to 10 d. The following type
and reference strains were used for biochemical assays, PCR
and DNA relatedness studies: *Bartonella bacilliformis*
ATCC 700693, *Bartonella clarridgeiae* strain 73 (Heller et al.,
1997), *Bartonella dushiae* ATCC 700133, *Bartonella
elizabethae* ATCC 49927T, *Bartonella henselae* ATCC
49882T, *Bartonella grahamii* ATCC 700132T, *Bartonella
koehlerae* ATCC 700693T, *Bartonella quintana* CIP 103739,
*Bartonella ramosissima* CIP 105476T, *Bartonella vinsonii*
*berkhoffii* ATCC 51672T and *Bartonella vinsonii* subsp.
*vinsonii*, ATCC VR-152T.

**Isolation of bacterial strains.** *Bartonella* isolates were cul-
tured from the blood of wild roe deer. Blood was drawn
within 45 min of death of the animal and frozen at −80 °C.
Thawed blood (200 µl) was streaked onto blood agar plates
made with a Columbia agar base supplemented with 5%
defibrinated sheep blood. Incubation of the plates was
performed at 37 °C in a moist atmosphere containing 5%
CO₂. The count (c.f.u. ml⁻¹) was determined for each blood
agar plate.

**Light microscopic examination.** Colony phenotypes were
observed using a binocular microscope at a magnification of
×5. Gram-staining of the cultured micro-organisms was
performed using standard methods (Chapin et al., 2000) and
examined with an oil-immersion objective at a magnification of
×1000.

**Electron microscopy.** Bacteria were prepared for electron
microscopy as described by Fussenegger et al. (1996). Briefly,
plate-grown bacteria were suspended in PBS, spread on a
water surface and absorbed to Formvar-coated nickel grids.
The grids were stained in 1% uranylacetate and air-dried.
The samples were viewed under a Zeiss M109 electron
microscope at 80 kV.

**Biochemical analysis.** Biochemical testing of the roe deer
isolates as well as of other *Bartonella* species was performed
essentially as described previously (Sander et al., 1997;
Sander, 1998; Chapin et al., 2000). *Bartonella* species are
biochemically inert in almost all conventional tests and
detection of enzyme activities was performed with test kits
which are primarily designed for the identification of
anaerobes. However, *Bartonella* species are not included in
their databases. The RapID ANA II system (Innovative
Diagnostic Systems) and Rapid ID 32 A (BioMérieux) were
used to test the activity of preformed bacterial enzymes in
accordance with the manufacturer’s instructions regarding
preparation, incubation and biochemical interpretation.
Leucyl-aryl-amidase activity was tested in the Api Strept
system (BioMérieux).

**Cellular fatty acid analysis.** Fatty acid methyl esters were
extracted from plate-grown bacteria and chromatographed
on a Hewlett Packard series II 5890 gas chromatograph as
described by Miller & Berger (1985). The molecules were
identified by using a computer-assisted comparison of their
retention times with a standard mixture (Microbial Identifi-
cation System).

**Protein profiles (SDS-PAGE analysis).** One-dimensional anal-
lytical SDS-PAGE was performed by the method of
Laemmli (1970) with a 12% separating gel and a 4.5% stacking
gel. Low-molecular-mass standards (Bio-Rad) were
used to estimate the molecular mass of the separated
proteins. The gel was stained with Coomassie blue R-250
(Serva).

**Chromosomal DNA preparation.** Bacterial DNA was
determined using Qiagen Genomic-tip according to the manu-
facturer’s instructions.

**Enterobacterial repetitive intergenic consensus (ERIC)-PCR.**
ERIC-PCR was performed as described by Sander et al.
(1998a). The primers ERIC1R (5'-ATGTAAGGTCCTG-
GGGATTGAC-3') and ERIC2 (5'-AAGTAAAGTGACTG-
GGGTTAGAGGG-3') used in this study have been de-
scribed by Versalovic et al. (1991). The reaction mixture
contained 8 ng µl⁻¹ bovine serum albumin (Sigma), 200 µM
each) of the four dNTPs, primers (117 nM each), 2 U Taq
polymerase (Pharmacia Biotech) and 100 ng genomic DNA
in 50 µl Tris borate/EDTA buffer. PCR amplification was
performed with initial denaturation (95 °C, 7 min), followed
by 30 cycles of denaturation (94 °C, 1 min), annealing
(40 °C, 1 min) and extension (65 °C, 8 min) with a single
final extension (65 °C, 16 min). The amplified products
(20 µl) were electrophoretically separated in a 1% agarose
gel at 120 V for 2 h in 0.5× Tris borate/EDTA buffer,
stained with ethidium bromide, visualized on an UV transilluminator and photographed with Polaroid 665 film.

**Amplification of the 16S rRNA and citrate synthase (gltA) genes.** Amplification of the 16S rRNA and citrate synthase genes was performed as described by Birtles & Raoult (1996) and Heller et al. (1997). Briefly, PCR amplification of an approximately 1500 bp DNA fragment was performed with the 16S rRNA gene eubacterial universal oligonucleotide primers P6 and Pc1544 (Heller et al., 1997). For the gltA gene, the oligonucleotide primers used for the amplification were CS140F and BhCS.1137n (Birtles & Raoult, 1996). Standard procedures to prevent sample DNA contamination were taken (Kwok & Higuchi, 1989). Negative controls were included in each experiment to check for the absence of cross-contamination between samples and between previously amplified products and field samples.

**Purification of the amplified product and DNA sequencing.** The amplified DNA fragments were purified by GeneClean glass beads (Bio101; Dianova). Sequencing reactions were performed by the AmpliTaq BigDyeTerminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Sequences were obtained with an ABI 377 DNA sequencer (Applied Biosystems). Sequencing of the complete 16S rRNA gene fragment was performed on coding and complementary strands, with four pairs of oligonucleotide primers as described previously (Heller et al., 1999). Partial sequencing of the gltA gene was performed with two pairs of oligonucleotide primer: BhCS.1137n and BhCS.781p (Norman et al., 1995), and pCHD205 (5′-TATAAGCCATAGCAG-CAAGAG-3′) and CS140F (Birtles & Raoult, 1996).

**Sequence analysis.** The sequences obtained were compared with similar sequences from other bacteria, including all *Bartonella* spp. contained in the EMBL/GenBank database. The sequences were aligned by using the CLUSTAL W program (Thompson et al., 1994; http://www.ebi.ac.uk/clustalw/) and multiple alignments were edited using Jalview (http://www.ebi.ac.uk/~michele/jalview/contents.html). Phylogenetetic trees were generated by the neighbour-joining method (Saitou & Nei, 1987) using CLUSTAL W and visualized by the software TREEVIEW (Page, 1996; http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

**DNA relatedness determination.** DNA relatedness experiments by the hydroxypatite method have been described by Brenner et al. (1982). DNA was labelled in vitro with [32P]dCTP using a nick-translation kit (Gibco). The temperature used for optimal hybridization was 55 °C and for stringent hybridization 70 °C, and divergence was calculated to the nearest 0-5%. All reactions were done at least twice.

**RESULTS**

**Roe deer population and bacterial culture**

Blood samples from five wild roe deer (designated R1T, R2, R3, R4 and R6) were collected aseptically within 45 min of death. The roe deer had been shot between September and December 1999 either in the Schönbuch Nature Park or the woodland area of the Spitzberg, both territories being situated in the vicinity of the city of Tübingen in south-western Germany. Before killing, all animals appeared healthy and no particular abnormalities in inner organs (i.e. liver, spleen, heart and lymph nodes) were observed during evisceration, especially in relation to *Bartonella* infection.

**Bacterial culture**

Plates streaked with blood from R2 remained sterile for more than 2 weeks, while on the plates streaked with the four remaining blood samples (of R1T, R3, R4 and R6), bacterial colonies appeared 4–6 d after inoculation and continued to grow for several days. The titre of colony-forming organisms in the various blood samples ranged from approximate 1000 to 10000 c.f.u. (ml blood)-1. Primary colonies had a characteristic firm, adherent, deeply invaginated, cauliflower-like morphology. After the first passage, a shiny, smooth colony phenotype appeared in addition (Fig. 1), which, due to more rapid growth, outgrew the primary colony phenotype upon multiple passages and was therefore used for further analysis.

**Phenotypic identification**

Light microscopic examination of the Gram-stained micro-organisms showed small, pleomorphic Gram-negative rods. They were often very short and slightly curved. Electron microscopic examination showed small rods with multiple, unipolar flagella (Fig. 2).

**Biochemical analysis**

In biochemical testing, the four new isolates tested were negative for catalase, oxidase, indole and urea hydrolysis. All the new strains were positive for leucyl-aryl-amidase activity (LAP) in the Api Strep system. In the RapID ANA II system the reaction profiles of the new isolates were 000 671, which is identical to that of *Bartonella henselae*, *Bartonella quintana* and several other *Bartonella* spp. In detail, enzymic hydrolysis of D-disaccharide (BLTS), L-arabinoside (aARA), D-galactoside (ONPG), D-glucoside (aGLU), D-galactoside (BGLU), L-fucoside (aFUC), D-glucosaminide (NAG), p-nitrophenylphosphate (PO4) and pyrrolidonyl (PYR) were negative. A strong...
positive reaction was seen in the enzymic hydrolysis of leucyl-glycine (LGY), glycine (GLY), arginine (ARG) and serine (SER). The hydrolysis of proline (PRO) and arginine (ARG) was less pronounced but clearly positive. Using this test system, *Bartonella clarridgeiae* and *Bartonella doshiae* are the only strains showing enzymic hydrolysis of N-glucosaminide (NAG).

The biotype code of the new isolates in the Rapid ID 32A system was 0000 0737 05 for strains R1<sup>T</sup>, R3 and R4, which are therefore indistinguishable from that of *Bartonella henselae*, *Bartonella quintana* and several other *Bartonella* species. Amino acid arylamidase activity was obtained with the following amino acids: arginine (ArgA), proline (ProA), leucyl-glycine (LGA), phenylalanine (PheA), leucine (LeuA), tyrosine (TyrA), alanine (AlaA), glycine (GlyA), histidine (HisA) and serine (SerA). In contrast, isolate R6 was lacking LGA and is therefore biochemically different from the other strains (code 0000 0337 05).

**Cellular fatty acid analysis**

The major fatty acids of the four new *Bartonella* isolates are cis-11-octadecanoic acid (C<sub>18:1ω7c</sub>), which accounted for 42.39–49.85% of total acids, octadecanoic acid (C<sub>18:0</sub>), 34.45–37.04% of total acids, and hexadecanoic acid (C<sub>16:0</sub>), 11.76–15.99% of total acids. Very small amounts of C<sub>12:0</sub> 3OH (0.55–0.69%) and C<sub>12:0</sub> 1OH (0.00–0.45%) were present but no cyclopropane acids were detected. These fatty acid patterns are similar to those observed for other *Bartonella* species (Sander, 1998).

**SDS-PAGE analysis**

Coomassie blue-stained SDS-polyacrylamide gels of total bacterial protein extracts of the four roe deer isolates showed three distinct protein profiles: R3 and R4 were similar, but R1<sup>T</sup> and R6 were different (Fig. 3). Distinct protein profiles were found for all the different *Bartonella* species tested (Fig. 3).

**ERIC-PCR**

The analysis of the four roe deer isolates by ERIC-PCR confirmed the results obtained by SDS-PAGE: strains R3 and R4 showed an identical banding pattern which was different from those obtained from strains R1<sup>T</sup> or R6. The fingerprint patterns of all other known *Bartonella* species are clearly different from those of the four roe deer isolates (Fig. 4).

**16S rRNA and gltA gene sequence analysis**

A 1446 bp region of the 16S rRNA gene sequence, corresponding to *Escherichia coli* positions 23–1521, was determined for the four roe deer isolates. Compared to strain R1<sup>T</sup>, strains R3, R4 and R6 had a single substitution of an adenosine by a cytosine residue at position 1021 (*E. coli* numbering system) (corresponding to 99.9% sequence identity). The sequences of the roe deer isolates were aligned with the 16S rRNA gene sequences of all other *Bartonella* species and with *Brucella abortus* as an outgroup. The GenBank accession numbers and strain designations for the
sequences used in the alignment are given in Table 1. Sequences in the alignment extending the fully aligned core region were deleted, resulting in a 1379 base sequence consisting of region 28–1459 (E. coli numbering). The multiple alignment was used to generate a phylogenetic tree (Fig. 5a), which indicated that the roe deer sequences were most similar to *Bartonella bacilliformis* (corresponding to 98.0% sequence identity with R1T).

![Fig. 4. DNA fingerprint analysis of the four new isolates and other Bartonella species by ERIC-PCR. Lanes: M, molecular mass markers; 1, negative control; 2, R1T; 3, R3; 4, R4; 5, R6; 6, Bartonella henselae; 7, Bartonella quintana; 8, Bartonella bacilliformis; 9, Bartonella elizabethae; 10, Bartonella clarridgeiae; 11, Bartonella alsatica; 12, Bartonella tribocorum; 13, Bartonella grahamii; 14, Bartonella doshiae; 15, Bartonella vinsonii spp. arupensis; 16, Bartonella vinsonii spp. berkoffii; 17, Bartonella vinsonii spp. vinsonii; 18, Bartonella koehlerae.](image-url)

The *gltA* gene sequence was determined for a region of 1013 bp (strain R1T), 1026 bp (strain R3), 1025 bp (strain R4) or 1034 bp (strain R6). A phylogenetic tree was generated based on a sequence alignment for the roe deer isolates together with all available *gltA* sequences from other *Bartonella* species which cover a similar size range, as well as *Sinorhizobium melloti* which served as the outgroup (Fig. 5b). The GenBank accession numbers and strain designations for the sequences used in the alignment are given in Table 1. Sequences in the alignment extending the shortest sequence included (*Bartonella grahamii*) were deleted, resulting in a 903 base sequence consisting of region 557–1459 of the *Sinorhizobium melloti* sequence. For the roe deer isolates this tree reflects a much higher sequence diversity in the *gltA* gene than observed for the 16S rRNA gene (sequence identities: 98.0% between R1T and R3/R4; 96.8% between R1T and R6; and 96.6% between R3/R4 and R6), while the four strains form a discrete cluster most closely related to *Bartonella bacilliformis* (86.6% sequence similarity with R1T).

**Table 1.** Strain designation and GenBank accession numbers for sequences in 16S rRNA gene and *gltA* gene trees

<table>
<thead>
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<th>Organism</th>
<th>Strain</th>
<th>16S rRNA</th>
<th>gltA</th>
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<td>R6</td>
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DNA hybridization studies

The results of DNA-relatedness studies are given in Table 2. The levels of DNA hybridization between the four roe deer isolates ranged from 76 to 100% under optimal hybridization conditions (55°C) and from 56 to 100% under stringent (70°C) hybridization conditions with divergence levels of 0–0.5°C. The percentage relatedness between the roe deer isolates and the type strains of other *Bartonella* spp. was less than 69% under optimal conditions and less than 43%
under stringent conditions, with divergence levels greater than 80%. Since no strains of *Bartonella taylorii*, *Bartonella talpae* or *Bartonella peromysci* are available, it was not possible to include these species in the DNA hybridization studies. Among the *Bartonella* species tested, *Bartonella henselae* was found to be closely related to the roe deer strains under optimal hybridization conditions (68% at 55°C), while *Bartonella bacilliformis* was found to be most closely related under stringent hybridization conditions (42% at 70°C).

**DISCUSSION**

*Bartonella* species have been identified as humanspecific pathogens (i.e. *Bartonella bacilliformis* and *Bartonella quintana*; Bass et al., 1997a) as well as important zoonotic agents (i.e. *Bartonella henselae* which naturally infects cats; Bass et al., 1997b). In the last 5 years, new *Bartonella* species have been isolated from a wide range of mammalian reservoirs, including rodents (Birtles et al., 1995; Heller et al., 1998), lagomorphs (Heller et al., 1999) and carnivores (Kordick et al., 1996), and a number of them have already been demonstrated to bear a significant zoonotic potential (Kordick et al., 1997; Ellis et al., 1999; Kerkhoff et al., 1999; Welch et al., 1999; Roux et al., 2000). In this study we have investigated the presence of bartonellae in roe deer. A previous study detected *Bartonella* DNA in more than 70% of the ticks collected from roe deer (Schouls et al., 1999), suggesting that this animal is frequently infected by bartonellae. Indeed, the recovery of four *Bartonella* strains from the blood of five roe deer from southwestern Germany indicates a high prevalence of bartonellae infection in this cervid. In North America, *Bartonella* isolates have been obtained in high frequency (90% prevalence of bacteraemia) from mule deer, another free-ranging wild cervid (Chang et al., 2000).

Primary cultures of the new isolates from roe deer were obtained on blood-enriched medium, as in the culture of other bartonellae. Primary colonies displayed a rough phenotype which changed to a smooth, fast-growing phenotype during passages, a dimorphism which has been previously reported for *Bartonella henselae* (Regnery et al., 1992). In contrast to the often slow growth of primary cultures of other *Bartonella* species, primary colonies of *Bartonella* isolates from roe deer have been obtained within 4–6 d of incubation.

Biochemical profiles cannot be used routinely and reliably for the differentiation of *Bartonella* species because of the relatively inert nature of bartonellae. Among the four roe deer isolates, only strain R6 could be distinguished from *Bartonella henselae* or other *Bartonella* species by biochemical testing using preformed enzyme assays. Whole-cell fatty acid analysis provides a useful tool for the identification of *Bartonella* at the genus level but is a poor method for differentiating species. Consistently, cellular fatty acid profiles of the four new isolates were found to be very similar to those of all other *Bartonella* species (high amounts of C\textsubscript{18:1} \(\omega 7\)c, C\textsubscript{18:0} and C\textsubscript{16:0}).

Repetitive element sequence-based PCR such as ERIC-PCR, has been successfully used for the identification of *Bartonella* strains on the species level as well to discriminate between isolates of the same species (Rodriguez-Barradas et al., 1995; Sander et al., 1998a). Using ERIC-PCR, we obtained from the four strains three different banding patterns, which were clearly different from all other *Bartonella* species. Three distinct protein profiles of the four new isolates were also found by SDS-PAGE. Consistently, DNA sequence analysis of the *gltA* gene again revealed significant heterogeneity within the three distinct sequences. In contrast, 16S rRNA sequences were less divergent, displaying a single base substitution in strain R1\textsuperscript{T} compared with the three others. These results provide evidence, not only for a clear distinction of the four strains from previously described species, but also for a significant heterogeneity among these strains, isolated from animals shot within an area of less than
Table 2. DNA relatedness of Bartonella isolates from roe deer to other Bartonella species

<table>
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<th>R6</th>
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<td>Bartonella koehlerae</td>
<td>59</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Bartonella elizabethae</td>
<td>56</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Bartonella alsatica</td>
<td>59</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Bartonella tribocorum</td>
<td>63</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Bartonella bacilliformis</td>
<td>62</td>
<td>8 5</td>
<td>42</td>
</tr>
</tbody>
</table>

ND, Not determined.

* Relative binding ratio [(percentage heterologous DNA bound to hydroxapatite/percentage homologous DNA bound by hydroxapatite) × 100] at 55 °C.

† Divergence (D) is the decrease in thermal stability (in degrees Celsius) of heterologous DNA duplexes compared with those of homologous DNA duplexes.

‡ Relative binding ratio [(percentage heterologous DNA bound to hydroxapatite/percentage homologous DNA bound by hydroxapatite) × 100] at 70 °C.

100 km². Although differences between strains belonging to one species have previously been noted for other Bartonella species (Sander et al., 1998b), the heterogeneity observed in the roe deer isolates appears to be very prominent. However, the limited sample size of this study did not allow epidemiological observations. Further studies need to be conducted to determine the heterogeneity of Bartonella isolates among roe deer populations.

DNA–DNA hybridization remains the method of choice for defining a species. It has been recommended that a species be considered to consist of strains whose DNAs are 70% or more related under optimal DNA reassociation conditions and 55% or more related under stringent DNA reassociation conditions, with 5% or less divergence within related sequences (Brenner et al., 1982; Wayne et al., 1987). According to these conditions, all four isolates belong to a single new species, although the strains are so divergent that we could have described two different subspecies (i.e. for R1^T and for R3, R4 and R6) as has been done previously in the genus Bartonella (Kordick et al., 1996; Welch et al., 1999). This novel species is proposed and described below. Based on stringent DNA hybridization and phylogenetic trees obtained for the 16S rRNA and the gltA gene sequences, the new Bartonella species is most closely related to Bartonella bacilliformis. Moreover, Bartonella bacilliformis together with Bartonella claridgeiae are the only described Bartonella species which carry flagella as described here for all the roe deer isolates. Regarding the relatedness of the new Bartonella species to Bartonella bacilliformis as well as its considerable heterogeneity we speculate that this pathogen not only has the potential to cause zoonosis (i.e. in hunters and forestry personnel who are exposed to the blood of roe deer or who are infested by ticks), but also that variants may adapt to humans, eventually developing into a human-specific pathogen such as Bartonella bacilliformis.

Description of Bartonella schoenbuchii sp. nov.

Bartonella schoenbuchii (schoen.buch′i.i. N.L. gen. n. schoenbuchii from Schönbuch, a nature park near Tübingen in south-west Germany where most of the roe deer analysed in this study were shot).

Growth obtained on Columbia agar base supplemented with 5% defibrinated sheep blood in a moist atmosphere containing 5% CO₂. Colonies appear in primary culture within 4–6 d as firm adherent, deeply invaginated, cauliflower-like colonies which upon passaging may switch to a more rapidly growing, shiny and smooth colony phenotype. The Gram-negative, monopolar politrichous flagellated rods are approximately 1–2 × 0.5 μm, aerobic and negative for oxidase, indole, catalase and urea hydrolysis. The code for preformed enzymes obtained in the RapID ANA II
system is 000 671. The biotype code using Rapid ID 32A was 0000 0737 05 or 0000 0337 05, depending on the strain analysed. The major fatty acids are cis-11-octadecanoic acid (C\textsubscript{18:1} \(\omega 7c\)), octadecanoic acid (C\textsubscript{18:0}) and hexadecanoic acid (C\textsubscript{16:0}), similar as observed for other Bartonella species. The four strains thus far isolated from the blood of healthy roe deer display phenotypic and genotypic differences but belong to a single new species. This new species is distinguished from previously known Bartonella species by the total protein profiles in SDS-PAGE, the banding patterns in the ERIC-PCR, the gltA and 16S rRNA gene sequences, and by whole-DNA hybridization analysis. Type strain is R1\textsuperscript{T}, isolated from the blood of a 4-month-old female wild roe deer. Deposited in the National Collection of Type Cultures as NCTC 13165\textsuperscript{T} and the Swiss National Science Foundation (SNF 3100-061777.00/1).

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