Transmission and Maintenance Cycle of *Bartonella quintana* among Rhesus Macaques, China

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We detected Bartonella quintana in 48.6% of captive rhesus macaques from an animal facility in Beijing, China. Prevalence of infection increased over the period of observation. Our findings suggest that macaques may serve as reservoir hosts for B. quintana and that Pedicinus obtusus lice might act as efficient vectors.

Bartonella quintana is a vector-transmitted, hemotropic, and extremely fastidious gram-negative bacterium. Infection with *B. quintana* has been recognized to cause a broad spectrum of disease, including trench fever, chronic bacteremia, endocarditis, and bacillary angiomatosis (*1–4*). Humans are the primary reservoir host for *B. quintana*, which, unlike most other *Bartonella* species, lacks an identified animal reservoir, although some recent reports have found *B. quintana* in dogs and in cynomolgus and rhesus macaques (*5–7*). Almost 60 years ago, rhesus macaques were able to be experimentally infected with *B. quintana* (*8*). However, nonhuman primates have not been shown to support long-term maintenance, multiplication, and transmission of this pathogen, all of which would be expected if these animals were to act as reservoir species. Observations of monkey ectoparasites transmitting *B. quintana* between nonhuman primates or infecting humans have also not been reported.

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The Study

A laboratory animal surveillance program intended to screen for the presence of adventitious pathogens was performed at the Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing, China. Four blood samples from 10 captive-bred rhesus macaques (*Macaca mulatta*) were presumed to be infected with *Bartonella* spp. according to Giemsa-stained smears and transmission electron microscopy (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/19/2/12-0816-Techapp1.pdf). Further PCR and sequence analysis of 3 gene targets (internal transcribed spacer [ITS], *gltA*, and *rnp*B) confirmed the existence of *B. quintana* in the 4 parasite-positive macaques (online Technical Appendix Table 1). In addition, *B. quintana* was successfully isolated from the 4 monkeys by blood plating.

During the 36-day period of observation, 3 screening tests of the 10 macaques showed an increasing prevalence of *B. quintana*: 4 were found positive at day 1, 7 positive at day 15, and all 10 positive at day 35 (Figure 1, panel A). Close examination of the monkeys revealed no skin scratch or wound indicative of direct contact between them.

Examination for ectoparasites at the last day of observation (day 36) revealed that all 10 monkeys were infested with lice (mean 10.3 lice/monkey, range 4–28 lice). Lice from each infested monkey were combined in 2 pools. *B. quintana* was identified in all pools of lice by PCR selective for ITS, *glt*A, and *rnp*B. Partial Cytb sequence (660-bp) of the louse was obtained (GenBank accession no. JX070558) (online Technical Appendix Table 1); phylogenetic analysis of Cytb identified the louse as a relative of lice of the genus *Pedicinus* (Figure 2, panel A). By means of stereomicroscopy, the louse was then identified as *Pedicinus obtusus* (Figure 1, panel B), a macaque-specific ectoparasite, according to morphologic criteria (*9,10*).

Of the 60 rhesus macaques (27 male, 33 female) housed in 5 other rooms in individual cages in the same facility, an additional 30 were found to be positive for *B. quintana* by PCR. *B. quintana* prevalence among sexually immature macaques was higher than that among sexually mature macaques, but this difference was not significant (29/54 [53.7%] vs. 1/6 [16.7%], respectively; $p = 0.195$). *B. quintana* prevalence among male macaques was similar to that among females (15/27 [55.6%] vs. 15/33 [45.5%], respectively).

Nucleotide sequences of ITS (123-bp), *gltA* (539-bp), and *rnpB* (336-bp) from all macaques and pools of lice were identical; they differed from from those of *B. quintana* strain Toulouse by1–3 bp . Phylogenetic markers *rnpB*, 16S rRNA, and 23S rRNA (*11*) were amplified and sequenced from the strain identified in this study

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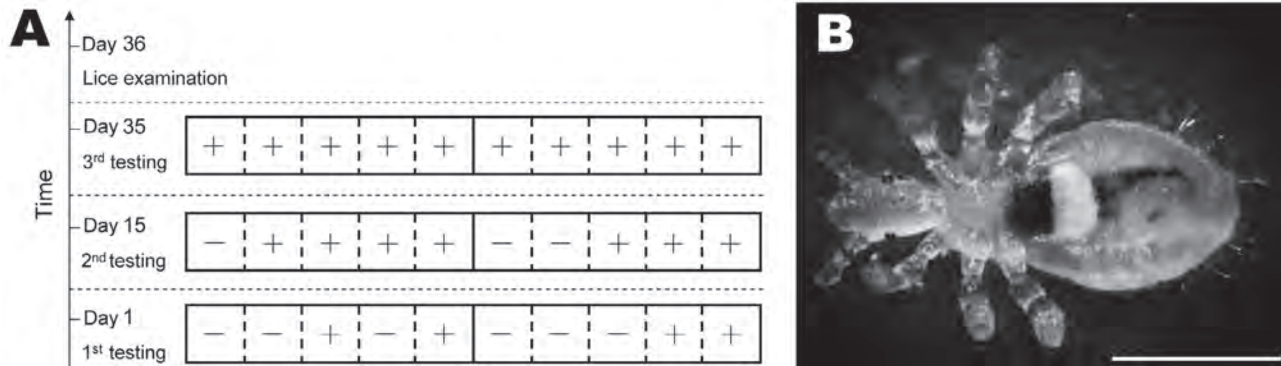

Figure 1. Monitoring surveillance of *Bartonella quintana* infection in macaques and identification of lice. A) Macaques were housed in linked cages (squares); dashed lines indicate wired net enabling direct contact between macaques, and solid line indicates wall that does not enable contact between macaques. +, positive result for PCR; –, negative result for PCR. B) Stereomicroscope image of a *Pedicinus obtusus* louse, a macaque-specific ectoparasite characterized by a slender body ≈1.0–3.0 × 0.5–1.0 mm; long, sharp claws in distal end of 6 legs of the same length; and a plurality of rows of small hairs on both sides of the abdomen. Scale bar indicates 500 μm.

(RM-11) (online Technical Appendix Table 1). Phylogenetic analysis of their combined sequence alignment placed the RM-11 strain on a separate branch along with the strain of *B. quintana* from a cynomolgus macaque and in the same clade as strains from patients in Europe who had trench fever (strains Toulouse and Fuller) (Figure 2, panel B).

To evaluate the ability of the isolate to cause disease, we intravenously inoculated 4 *Bartonella* spp.–negative rhesus macaques with *B. quintana* isolated from a blood sample of a macaque from this study and twice passaged on agar (detailed methods described in the online Technical Appendix). Bacteremia reached a peak in 1 monkey on day 7 postinoculation (160 CFU/mL), in 2 monkeys on day 14 postinoculation (290 and 240 CFU/mL), and in 1 monkey on day 42 postinoculation (240 CFU/mL). Bacteremia then dropped to below a detectable level after 15 weeks postinoculation for all monkeys (online Technical Appendix Figure 2). A relapsing pattern of bacteremia was observed during the experiment. Rectal temperature, hemogram, and blood biochemistry results for the 4 monkeys remained within normal limits.

The animal facility employees who had direct contact with monkeys during cage cleaning and feeding activities were tested for *B. quintana* infection. Paired serum samples collected at 2 time points 3 months apart were tested for IgG against *B. quintana* by indirect immunofluorescence assay, as described (*12*). The baseline serum samples were all negative at a dilution of 1:64. Among the serum samples collected 3 months later, 3 had IgG titers of 256, 1 had a titer of 512, and 4 were negative. For all blood samples collected at the 2 time points, PCR detection, blood-smear staining, and blood culture for *Bartonella* spp. were negative. Analysis of questionnaires revealed that all 4 of the workers with evidence of seroconversion reported lice exposure; 2 of them were scratched or bitten by monkeys.

Conclusions

We demonstrated high prevalence of *B. quintana* in a colony of rhesus macaques and postulated the transmission among macaques by *P. obtusus* lice. Our findings suggest that macaques are susceptible to *B. quintana* infection and can sustain vector infection and subsequent transmission. In addition, rhesus macaques showed long-lasting chronic bacteremia without apparent clinical abnormalities after experimental inoculation, suggesting a high level of adaptation of the pathogen to macaques.

It is unknown how the macaques were initially exposed to infected lice or how the lice became infected with *B. quintana.* We postulate that the lice became infected with *B. quintana* from an infected macaque and thereafter acted as efficient vectors among the rest of the macaques in the colony. We cannot completely exclude the possibility that transmission occurred through direct contact between macaques within the colony; however, we did not observe any skin scratches or wounds on the animals.

Four workers involved with care of the macaques showed seroconversion to antigens derived from our strain. This finding may relate to exposure to the *P. obtusus* lice found on macaques in the animal facility; however, we cannot exclude the possibility of direct contact with these animals as the mode of *Bartonella* spp. transmission because information obtained from the questionnaires indicates that 2 of the 4 seropositive workers were scratched or bitten by the colony's macaques. No clinical signs were observed from these persons, indicating an asymptomatic course of *B. quintana* infection, which has been frequently reported in other studies (*1,13*). However, we cannot exclude the possibility that bacteremia continued for an extended time in humans, because the animal care personnel were sampled only 2 times, so we might have missed bacteremia of short duration. Bacterial levels obtained after monkey

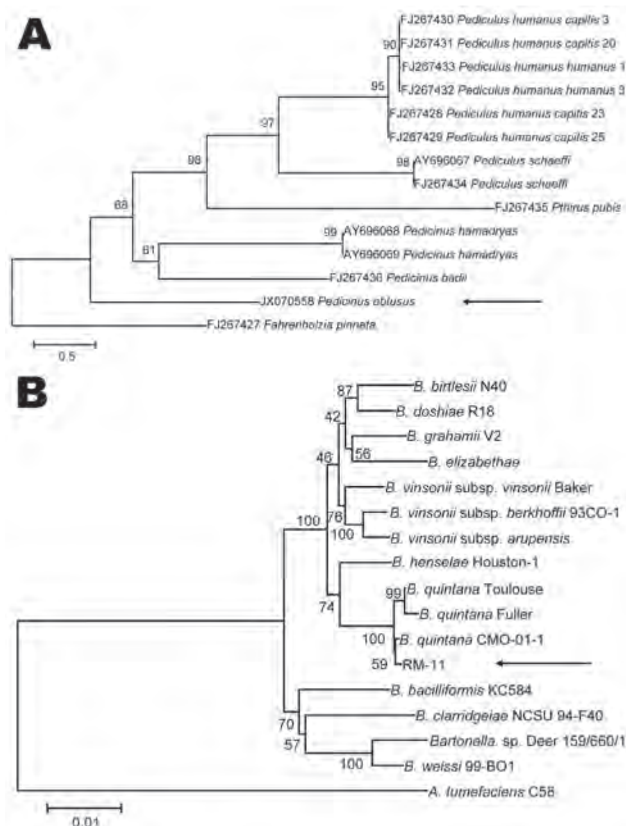

Figure 2. Phylogenetic analyses of louse species and *Bartonella* spp. A) Phylogenetic tree of louse species based on the partial Cytb sequence (364-bp), obtained by using the neighbor-joining method with maximum composite likelihood analysis and bootstrap analysis of 1,000 replicates. Arrow indicates the *Pedicinus obtusus* louse identified in this study. The tree was rooted with the louse species *Fahrenholzia pinnata*. Numbers shown at each node indicate percentage of replicates that reproduced the topology of each clade. Scale bar indicates estimated evolutionary distance of 0.5 substitutions per position. B) Phylogenetic tree of *Bartonella* spp. based on the combined RNase P RNA, 16S, and 23S rRNA sequence alignment (4131-bp), obtained by using the same analytical method as described in panel A. Arrow indicates the RM-11 isolate. The tree was rooted with the louse species *Agrobacterium tumefaciens*. The GenBank accession numbers of *Bartonella* strains used for phylogenetic analysis are shown in online Technical Appendix Table 2 (wwwnc.cdc.gov/EID/article/19/2/12-0816-Techapp1.pdf). Scale bar indicates estimated evolutionary distance of 0.01 substitutions per position.

inoculation was low compared with levels in the initial inoculum, a finding similar to that of a previous study (*14*).

In summary, our findings suggest that the macaques might serve as reservoir hosts for *B. quintana* and that lice might act as efficient vectors. Our data also indicate that macaques could be a source for human infection with *B. quintana*. Further research is needed to understand the underlying mechanism of *B. quintana* transmission by the *P. obtusus* louse.

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References

1. Brouqui P, Lascola B, Roux V, Raoult D. Chronic *Bartonella quintana* bacteremia in homeless patients. N Engl J Med. 1999;340:184–9. http://dx.doi.org/10.1056/NEJM199901213400303
2. Drancourt M, Mainardi JL, Brouqui P, Vandenesch F, Carta A, Lehnert F, et al. *Bartonella* (Rochalimaea) *quintana* endocarditis in three homeless men. N Engl J Med. 1995;332:419–23. http://dx.doi.org/10.1056/NEJM199502163320702
3. Klein JL, Nair SK, Harrison TG, Hunt I, Fry NK, Friedland JS. Prosthetic valve endocarditis caused by *Bartonella quintana.* Emerg Infect Dis. 2002;8:202–3. http://dx.doi.org/10.3201/eid0802.010206
4. Koehler JE, Sanchez MA, Garrido CS, Whitfeld MJ, Chen FM, Berger TG, et al. Molecular epidemiology of *Bartonella* infections in patients with bacillary angiomatosis-peliosis. N Engl J Med. 1997;337:1876–83. http://dx.doi.org/10.1056/NEJM199712253372603
5. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis. 2006;12:389–94. http://dx.doi.org/10.3201/eid1203.050931
6. O'Rourke LG, Pitulle C, Hegarty BC, Kraycirik S, Killary KA, Grosenstein P, et al. *Bartonella quintana* in cynomolgus monkey (*Macaca fascicularis*). Emerg Infect Dis. 2005;11:1931–4. http://dx.doi.org/10.3201/eid1112.030045
7. Huang R, Liu Q, Li G, Li D, Song X, Birtles RJ, et al. *Bartonella quintana* infections in captive monkeys, China. Emerg Infect Dis. 2011;17:1707–9. http://dx.doi.org/10.3201/eid1709.110133
8. Mooser H, Weyer F. Experimental infection of *Macacus rhesus* with *Rickettsia quintana* (trench fever). Proc Soc Exp Biol Med. 1953;83:699–701.
9. Kim KC, Ludwig HW. The family classification of the Anoplura. Syst Ent. 1978;3:249–84. http://dx.doi.org/10.1111/j.1365-3113.1978.tb00120.x
10. Jin S, Rao J, Liu X, Wen Y, Lian J. Diagnosis and therapy of *Pedicinus obtusus* Infection on *Macaca mulatta.* Chin J Comp Med. 2006;16:424–5.
11. Pitulle C, Strehse C, Brown JW, Breitschwerdt EB. Investigation of the phylogenetic relationships within the genus *Bartonella* based on comparative sequence analysis of the *rnpB* gene, 16S rDNA and 23S rDNA. Int J Syst Evol Microbiol. 2002;52:2075–80. http://dx.doi.org/10.1099/ijs.0.02281-0
12. Kosoy MY, Regnery RL, Tzianabos T, Marston EL, Jones DC, Green DE, et al. Distribution, diversity, and host specificity of *Bartonella* in rodents from the southeastern United States. Am J Trop Med Hyg. 1997;57:578–88.
13. Bass JW, Vincent JM, Person DA. The expanding spectrum of *Bartonella* infections: I. Bartonellosis and trench fever. Pediatr Infect Dis J. 1997;16:2–10. http://dx.doi.org/10.1097/00006454-199701000-00003

14. Zhang P, Chomel BB, Schau MK, Goo JS, Droz S, Kelminson KL, et al. A family of variably expressed outer-membrane proteins (Vomp) mediates adhesion and autoaggregation in *Bartonella quintana.* Proc Natl Acad Sci U S A. 2004;101:13630–5. http://dx.doi.org/10.1073/pnas.0405284101

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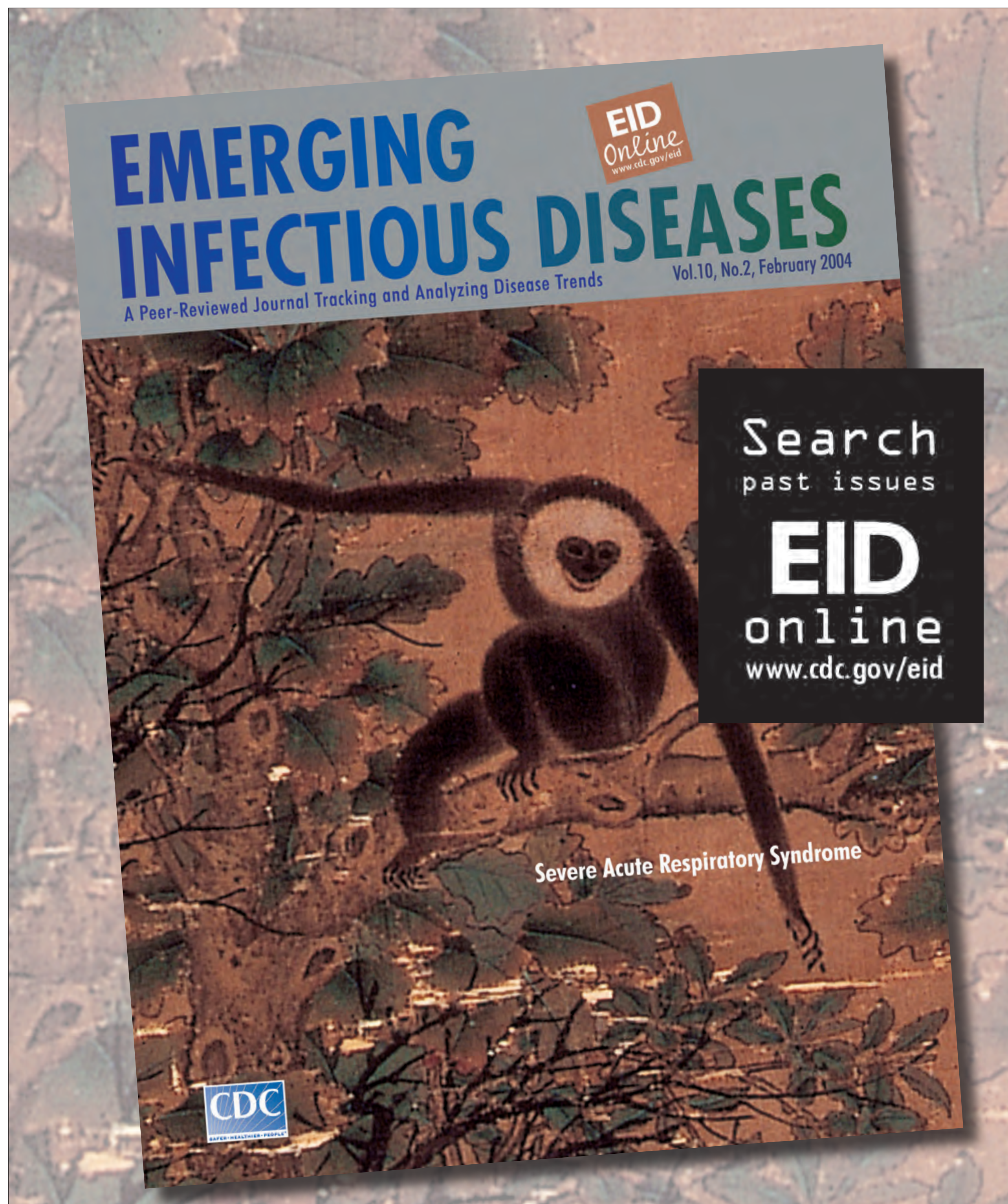

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Technical Appendix

Bartonella DNA detection

PCR analyses were performed at the Beijing Institute of Microbiology and Epidemiology. Briefly, DNA was extracted from blood specimens using QIAmp DNA Mini Kit (Qiagen). PCR targeting the 16S-23S internal transcribed spacer region (ITS), citrate synthase (*gltA*), and RNase P RNA (*rnpB*) genes were carried out (Technical Appendix Table 1), with *B. grahamii* as a positive control and sterile deionized water as a negative control. A positive result was determined only when all three targets were amplified. For positive samples, 16S rDNA-encoding and 23S rDNA-encoding genes were additionally amplified for further phylogenetic characterization (Technical Appendix Table 1). Short amplified DNA fragments (<800-bp) were directly sequenced in both directions, and long DNA fragments were cloned into pGEM-T easy vector system (Promega) for sequencing on an automated DNA sequencer (3730 DNA Sequencer, Applied Biosystems). To reduce contamination, DNA extraction reagent setup and amplification were performed in separate rooms. Certified DNA/RNase-free filter barrier tips were used to prevent aerosol contamination.

Cytb gene amplification

Pooled (2–14 lice/pool) lice samples were homogenized in sterile phosphate buffered saline with a Bullet Blender (NextAdvance Inc., Averill Park, NY, USA). DNA was extracted

from the homogenates using DNeasy Tissue Kit (Qiagen) according to manufacturer's instructions. For phylogenetic characterization of the louse, a portion of cytochrome b gene (Cytb) was amplified and sequenced with primers Cytb-f and Cytb-r (Appendix Table 1).

Inoculation of naive rhesus macaques

Four rhesus macaques confirmed to be *Bartonella*-negative by morphologic examination, blood test by nested PCR and serum test by indirect immunofluorescence assay were selected (all tests repeated after a week's interval), deloused, and held in a clean room for 7 days before the inoculation. The macaques were intravenously inoculated with isolate of *B. quintana* that was originally isolated from a blood sample of a macaque from this study and twice passaged on agar. Peripheral blood was collected post inoculation weekly in EDTA vacuum tubes. The frozen-thawed blood specimens were plated in duplicates on chocolate agar. Colony forming units (CFUs) were counted on day 15 after plating. During the whole observation period, rectal morning temperature was taken daily; laboratory routine tests of hemogram (leukocyte, erythrocyte, lymphocyte, granulocytes, platelet, hemoglobin and hematocrit) and blood biochemistry (alkaline phosphatase, alanine transaminase, aspartate aminotransferase, lactate dehydrogenase, albumin, urea nitrogen, cholesterol, triglyeride, glucose, creatine kinase, creatinine) were performed every third day.

IFA serology

Colonies of *B. quintana* harvested after 5 days of the growth on a chocolate agar plate were inoculated onto Vero E6 cells. After 3 days, the infected cells were harvested for antigen preparation. Slides of culture cells were fixed in a 1:1 solution of acetone and methanol and prepared for indirect immunofluorescence assay (IFA). Briefly, all serum samples were diluted 1:64 in PBS, overlaid onto antigen-containing slides, incubated at 37°C for 30 minutes, washed, and incubated at 37°C for 30 minutes with goat antihuman immunoglobulin G conjugated with

fluorescein isothiocyanate. Positive samples were then tested with additional serial dilutions. A serum with antibodies against *B. quintana* (Euroimmun, Lubeck, Germany) was used as a positive control for IFA.

Reference

1. Jensen WA, Fall MZ, Rooney J, Kordick DL, Breitschwerdt EB. Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. J Clin Microbiol. 2000;38:1717–22. PubMed

2. Brouqui P, Lascola B, Roux V, Raoult D. Chronic *Bartonella quintana* bacteremia in homeless patients. N Engl J Med. 1999;340:184–9. PubMed http://dx.doi.org/10.1056/NEJM199901213400303

3. Birtles RJ, Raoult D. Comparison of partial citrate synthase gene (gltA) sequences for phylogenetic analysis of *Bartonella* species. Int J Syst Bacteriol. 1996;46:891–7. PubMed http://dx.doi.org/10.1099/00207713-46-4-891

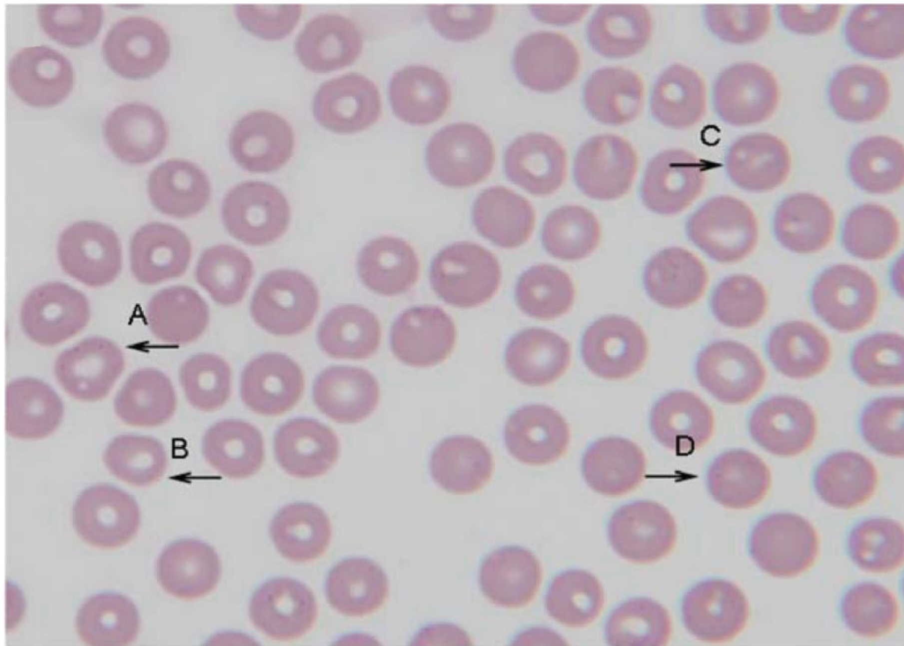
Technical Appendix Table 1. Nucleotide sequence of primers used for PCR analysis

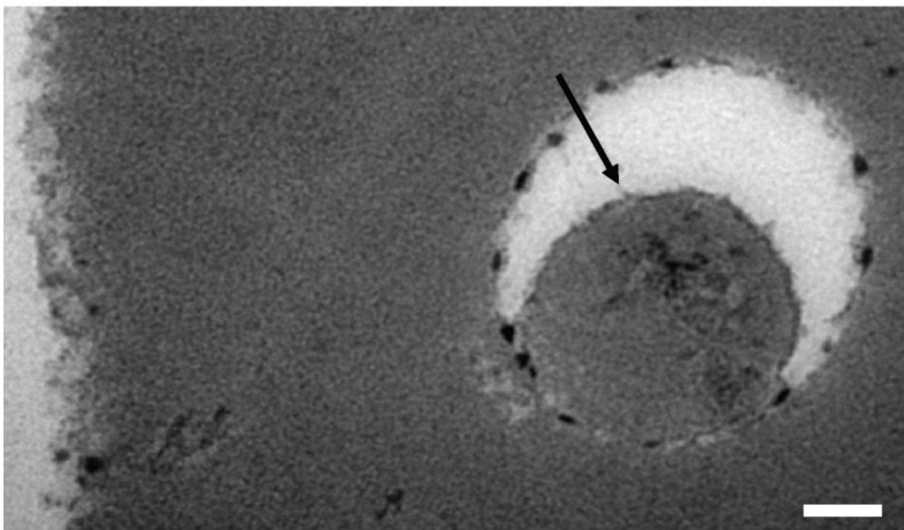
Target	Primer	Primer sequence (5′ → 3′)	Reference
ITS	302F	YCTTCGTTTCTCTTTCTTCA	(*1*)
	473R	AACCAACTGAGCTACAAGCC	
	311F	CTCTTTCTTCAGATGATGATCC	
	448R	GGATAAACCGGAAAACCTTC	
gltA	CS140f	TTACTTATGATCCKGGYTTTA	(*2*,*3*)
	CS 443	GCTATGTCTGCATTCTATCA	
	CS 979	TGCATGATTTTTGCACGTGG	
rnpB	rnpB-Fo	AGTCGGCTGGGCAACCGCGC	This study
	rnpB-Ro	GCCTGTAAGCCGGGTTCTGTA	
	rnpB-Fi	GCAAGTGAGGAAAGTCCG	
	rnpB-Ri	TGTAAGCCGGGTTCTGTA	
16S rDNA	16S-F1	ACTGTCTCATAATGAGGTAGAGGC	This study
	16S-R1	AGATTTCGGAAAGAATATGGCG	
	16S-F2	GATTTAGCGTCATATGCATGGTT	
	16S-R2	ATATGTTCTCGTCGATTCAAGC	
23S rDNA	23S-Fo	tttgtgagtgatgctctatgcg	This study
	23S-Ro	AGAAGCTGGTCTTTTCTGCTG	
	23S-Fi	ccataaccaccaagtcagcaa	
	23S-Ri	TCCTGGAGGTATCGGAAGTGA	
	23S-Fii	aagaccttacaatacacgcaatc	
	23S-Rii	CAAAGAATGCCGACAAACATTG	
Cytb	Cytb-f	GCTACTCATTATGARKCTTC	This study
	Cytb-r	TCTGGYTGRATATGAGGWGGWGT	

Technical Appendix Table 2. GenBank accession numbers of *Bartonella* strains used for phylogenetic analysis in this study*

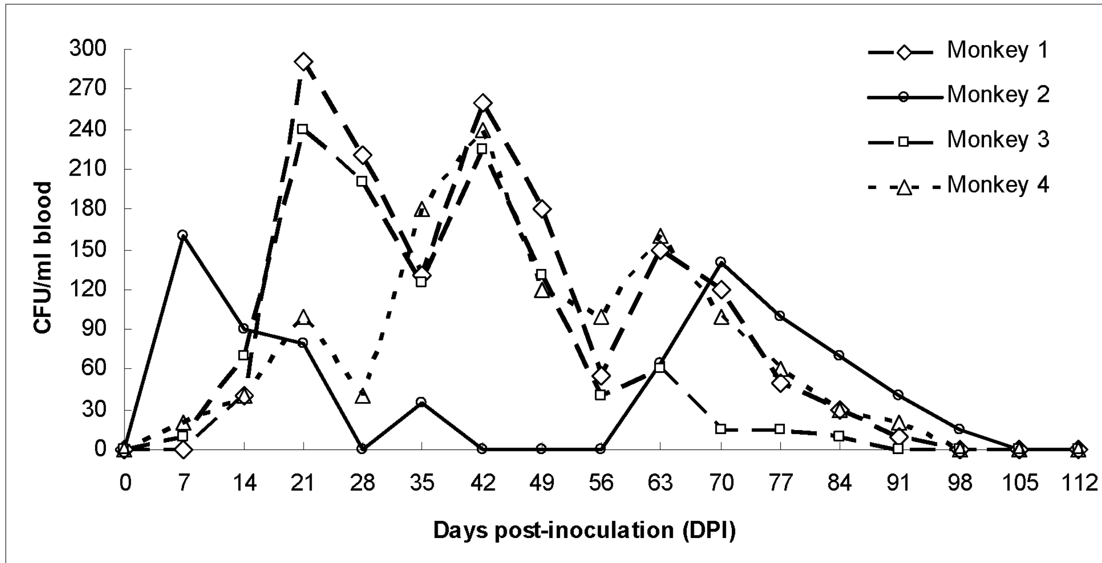
Species/strain	*rnpB*	16S rDNA	23S rDNA
B. bacilliformis KC584	AF440224	AF442955	L39095
B. birtlesii N40	AF441292	AF204274	AF410944
B. clarridgeiae NCSU 94-F40	AY033649	U64691	AF410938
B. doshiae R18	AF441294	Z31351	AF410939
B. elizabethae F9251	AY033770	L01260	AF410940
B. grahamii V2	AF441293	Z31349	AF410942
B. henselae Houston-1	AY033897	M73229	AF410943
B. quintana CMO-01–1	AY484594	AY484592	AY484593
B. quintana Fuller	AY033948	M11927	AF410946
B. quintana Toulouse	BX897700	BX897700	BX897700
B. Vinsonii subsp. *arupensis*	AF441295	AF214558	AF410937
B. vinsonii subsp. *vinsonii* Baker	AY033502	Z31352	AF411589
B. vinsonii subsp. *berkhoffii* 93CO-1	AF375873	L35052	AF410941
B. weissi 99-BO1	AF376050	AF291746	AF410947
Bartonella. sp. Deer 159/660/1	AF376051	AF373845	AF410945
RM-11	JQ314421*	JQ314414*	JQ314415*

*GenBank accession numbers for sequences of the *rnpB*, 16S rDNA, and 23S rDNA are listed. Sequences data of the strain isolated in the study were deposited in GenBank under the accession numbers indicated by asterisks.





Technical Appendix Figure 1. Top: Giemsa stain of thin-film blood smear. A) Suspected intraerythrocytic corpuscles; B) Suspected member-associated corpuscles; C) Stomatocyte; D) Spherostomatocyte with suspect intraerythrocytic corpuscles. Bottom: Observation of peripheral blood from rhesus macaque by transmission electron microscopy shows erythrocyte with vacuole-enclosed suspected organism. Scale bar indicates 100 nm.



Technical Appendix Figure 2. Timeline of bloodstream infections of strain RM-11 in 4 rhesus macaques. Blood cultures were performed in duplicate weekly for 112 days. CFU of *B. quintana* bacteria per ml of blood was counted on day 15 after blood plating.