

Prevalence and Genetic Diversity of *Bartonella* Species Detected in Different Tissues of Small Mammals in Nepal^{∇†}

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Bartonellae were detected in a total of 152 (23.7%) of 642 tissues from 108 (48.4%) of 223 small mammals trapped in several urban areas of Nepal. Based on *rpoB* and *gltA* sequence analyses, genotypes belonging to seven known *Bartonella* species and five genotypes not belonging to previously known species were identified in these animals.

Bartonella species are important zoonotic and vector-borne bacteria associated with an increasing array of emerging infections in humans and animals (6, 8, 21). Identification of new bartonellae, especially in animals that have possible contact with humans, can help to identify new human pathogens (12). Moreover, the close association between rodents and humans throughout the world makes the study of rodent-borne *Bartonella* essential to determine the extent to which rodents may serve as a source of human infections (7). We conducted a study on the molecular detection of *Bartonella* in different tissues of Nepalese small mammals.

In 1996, a total of 223 small mammals (38 *Bandicota bengalensis*, 3 *Mus musculus castaneus*, 90 *Rattus rattus brunneusculus*, and 92 *Suncus murinus* animals) were trapped in densely human populated urban areas in Nepal. Details on the trapped animals are given in Table 1. Lung, kidney, and liver tissue samples were collected and shipped to the Centers for Disease Control and Prevention (CDC), Fort Collins, CO. A total of 642 tissue samples were assessed for the presence of *Bartonella* DNA by *rpoB* and *gltA* sequencing as described previously (5, 23).

Bartonella was detected in 108 (48.4%) of 223 animals in Nepal: 10 (26.3%) of 38 *B. bengalensis* rats, 39 (43.3%) of 90 *R. rattus brunneusculus* rats, and 59 (64.1%) of 92 *S. murinus* shrews were infected with *Bartonella* (Table 1). The infection rate varied from 42 to 60% by province. The highest numbers of infected animals were in the Kathmandu and

Lalitpur districts. This was because *S. murinus* was the most commonly infected species and the highest numbers of animals of this species were trapped in these areas. Bandicoots were the most commonly infected subjects in Kathmandu, but fewer numbers of these animals ($n = 2$) were trapped in Bhaktapur and Lalitpur. Only three *M. musculus* mice were collected (only in Kathmandu) in this study, and none of these animals were positive for *Bartonella*. A total of 152 (23.7%) of 642 tissue samples were positive for *Bartonella*. Of the three tissue types, kidney samples (29.6%) and liver samples (25.6%) were more frequently infected with *Bartonella* than the lung samples (17.3%; $P = 0.03$ and 0.04 , respectively) (Table 2). DNA sequencing revealed 37 and 20 *rpoB* and *gltA* genotypes, respectively, of which 31 and 15 belonged to *B. rattimassiliensis*, *B. queenslandensis*, *B. elizabethae*, *B. tribocorum*, “*B. rochalimae*,” or “*B. phoceensis*” and the other 6 and 5 genotypes, respectively, were not genetically related to known bartonellae (Fig. 1 and 2). These novel genetic groups were found in *S. murinus* and *R. rattus brunneusculus* animals. The human pathogen *B. elizabethae* was detected in *R. rattus brunneusculus* rats ($n = 5$) and an *S. murinus* shrew ($n = 1$), and the human pathogen *B. rochalimae* was found in a *B. bengalensis* bandicoot ($n = 1$) and an *S. murinus* shrew ($n = 1$).

Our study reports the first molecular detection and findings on the prevalence of *Bartonella* in small mammals of Nepal and is also the first study to compare different organ tissues from the same animal for *Bartonella* detection. Previously, several reports were made on the occurrence of bartonellae in rodents and other small mammals in Asian countries, including Bangladesh (3), China (28), Indonesia (27), Japan (16), Laos (1), Taiwan (19), and Thailand (2, 7, 24). Recently, Bai et al. (3) reported that 63.2% of *B. bengalensis*, 32.3% of *R. rattus*, and 42.9% *S. murinus* animals collected in Dhaka, Bangladesh, were infected with *Bartonella*, and the isolates from these animals were genetically related to

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TABLE 1. Prevalence of *Bartonella* in small mammals trapped in different districts of Nepal

District ^a	Trap area ^b	No. of <i>Bartonella</i> -infected animals/no. of animals examined (%)					Subtotal	Total
		<i>Bandicota bengalensis</i>	<i>Mus musculus castaneus</i>	<i>Rattus rattus brunneusculus</i>	<i>Suncus murinus</i>			
Bhaktapur	Bhelukhel			3/5 (60)			3/5 (60)	
	Bhelukhel			1/1 (100)			1/1 (100)	
	Pode Tole	0/2 (0)		4/8 (50)	0/1 (0)		4/11 (36.4)	
	NK			4/12 (33.3)	4/9 (44.4)		8/21 (38.1)	
Subtotal		0/2 (0)	0/0 (0)	12/26 (46.2)	4/10 (40)		16/38 (42.1)	
Kathmandu	Hyumata			2/2 (100)	2/3 (66.7)		4/5 (80)	
	Inakha Tole				3/3 (100)		3/3 (100)	
	Kalimati	2/6 (33.3)	0/1 (0)	1/5 (20)	7/12 (58.3)		10/24 (41.7)	
	Lagan Tole	0/4 (0)		2/3 (66.7)	3/5 (60)		5/12 (41.7)	
	Sabalbahal	7/21 (33.3)	0/1 (0)	2/6 (33.3)	7/14 (50)		16/42 (38.1)	
	Teku	1/4 (25)	0/1 (0)	4/13 (30.8)	1/1 (100)		6/19 (31.6)	
	Tokha (suburb)			1/1 (100)			1/1 (100)	
	NK			1/2 (50)	15/25 (60)		16/27 (59.3)	
Subtotal		10/35 (28.6)	0/3 (0)	13/32 (40.6)	38/63 (60.3)		61/133 (45.9)	
Lalitpur	Bakhar			4/4 (100)			4/4 (100)	
	Bakhar Thati			0/3 (0)			0/3 (0)	
	Lagankhel			1/1 (100)			1/1 (100)	
	Patan				2/2 (100)		2/2 (100)	
	Sabalbahal	0/1 (0)					0/1 (0)	
Subtotal		0/1 (0)	0/0 (0)	14/32 (43.8)	17/19 (89.5)		31/52 (59.6)	
Total		10/38 (26.3)	0/3 (0)	39/90 (43.3)	59/92 (64.1)		108/223 (48.4)	

^a The following *Bartonella* species were detected: in Bhaktapur, *B. cooperplainsensis*, *B. elizabethae*, *B. queenslandensis*, and a novel *Bartonella* species; in Kathmandu, *B. cooperplainsensis*, *B. elizabethae*, *B. queenslandensis*, *B. rochalimae*, *B. tribocorum*, and a novel *Bartonella* species; and in Lalitpur, *B. cooperplainsensis*, *B. elizabethae*, *B. phoceensis*, *B. queenslandensis*, *B. rattimassiliensis*, *B. tribocorum*, and a novel *Bartonella* species.

^b NK, not known.

Bartonella isolates found in America and Europe. Our study produced similar results in terms of the infectivity of the animals and the specificity of bartonellae. It is evident from all these studies that *B. elizabethae*, *B. phoceensis*, *B. rattimassiliensis*, *B. rochalimae*, and *B. tribocorum* are being circulated among small animals in Asia. Furthermore, *B. cooperplainsensis* and *B. queenslandensis*, found initially in Australia, were also detected in Nepal (this study) and Bangladesh (3), suggesting that the origin of these species might be Australasia.

The prevalence of *Bartonella* in Nepal was comparable with prevalence rates on other continents: in Africa, 44% in

South Africa (22) and 24% in the Democratic Republic of Congo and Tanzania (V. A. K. B. Gundi et al., unpublished data); 42% in North America (18); and in Europe, 17% in France (12), 17% in central Sweden (14), 28% in southwestern Spain (20), 28% in Denmark (11), 31% in Greece (25), 31% in northeast Poland (26), 40% in Slovenia (17), and 64% in the United Kingdom (4). In a recent study, 29% prevalence was recorded in Australia (13). Such high rates of *Bartonella* prevalence are significant in the context of human health, because the synanthropic mammals harboring pathogenic microorganisms are often found in biotopes

TABLE 2. Detection of *Bartonella* DNA in different tissues of small mammals in Nepal

Species	No. of animals infected/ no. of animals tested (%)	No. of <i>Bartonella</i> -infected tissue samples/no. of tissue samples examined (%)			
		Kidney	Liver	Lung	Total
<i>Bandicota bengalensis</i>	10/38 (26.3)	5/38 (13.2)	2/38 (5.3)	5/18 (27.8)	12/94 (12.8)
<i>Mus musculus castaneus</i>	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/9 (0)
<i>Rattus rattus brunneusculus</i>	39/90 (43.3)	12/90 (13.3)	32/90 (35.6)	15/89 (16.9)	59/269 (21.9)
<i>Suncus murinus</i>	59/92 (64.1)	49/92 (53.3)	18/92 (19.6)	14/86 (16.3)	81/270 (30)
Total	108/223 (48.4)	66/223 (29.6)	52/203 (25.6)	34/196 (17.3)	152/642 (23.7)

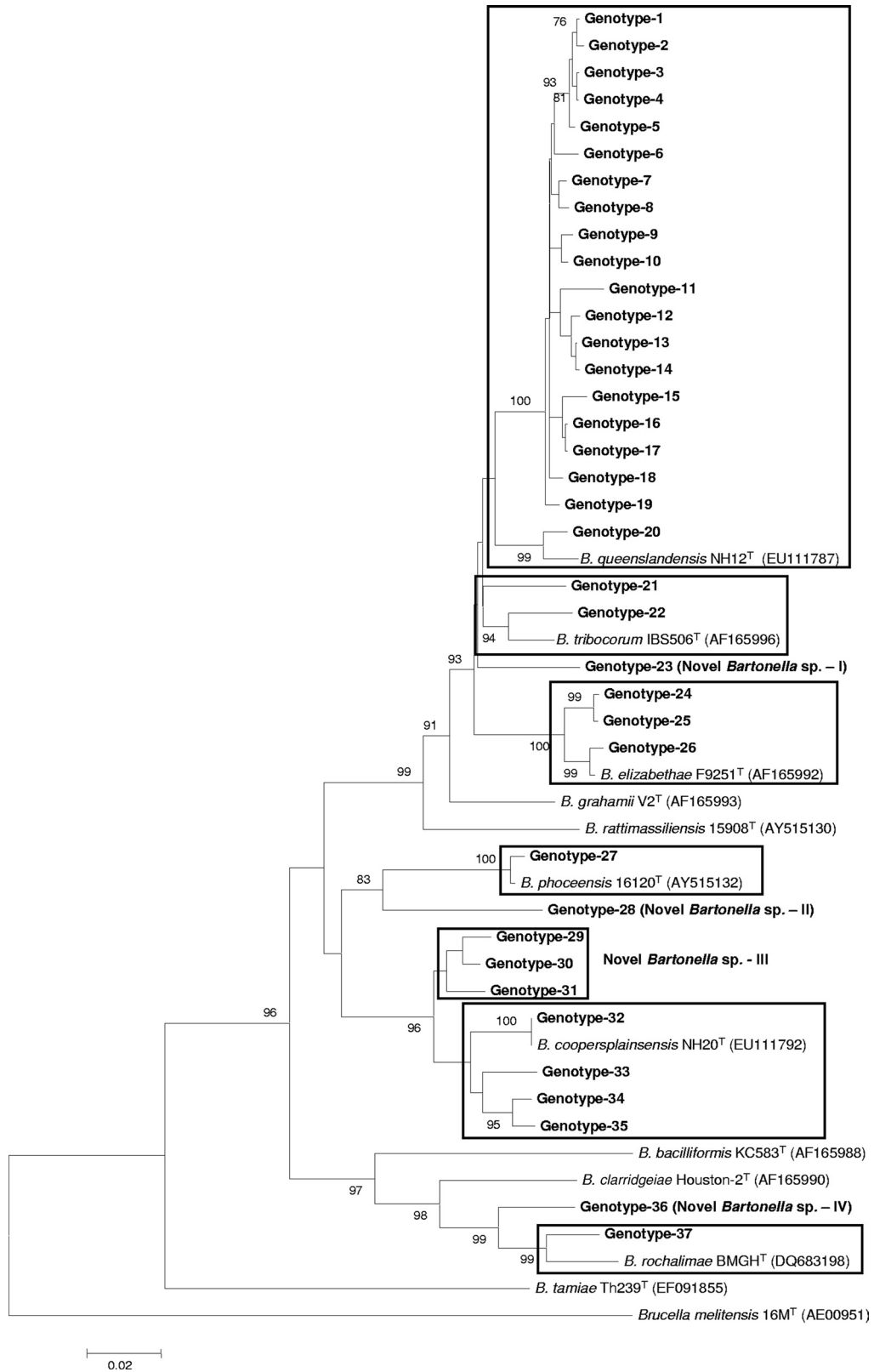


FIG. 1. Phylogenetic classification of *Bartonella* genotypes detected in small mammals of Nepal based on *rpoB* gene sequences. The phylogram was constructed by the neighbor-joining method with the Kimura 2-parameter model. Only the bootstrap values above 70% obtained from 1,000 replicates are given. The 76 different sequences of *rpoB* from small mammals of Nepal were classified into seven clusters. The GenBank accession numbers for reference sequences are given in parentheses. Only known reference *Bartonella* sequences without gaps and missing data are included in the phylogram. The *rpoB* gene sequence of *Brucella melitensis* 16M^T was included as an out-group.

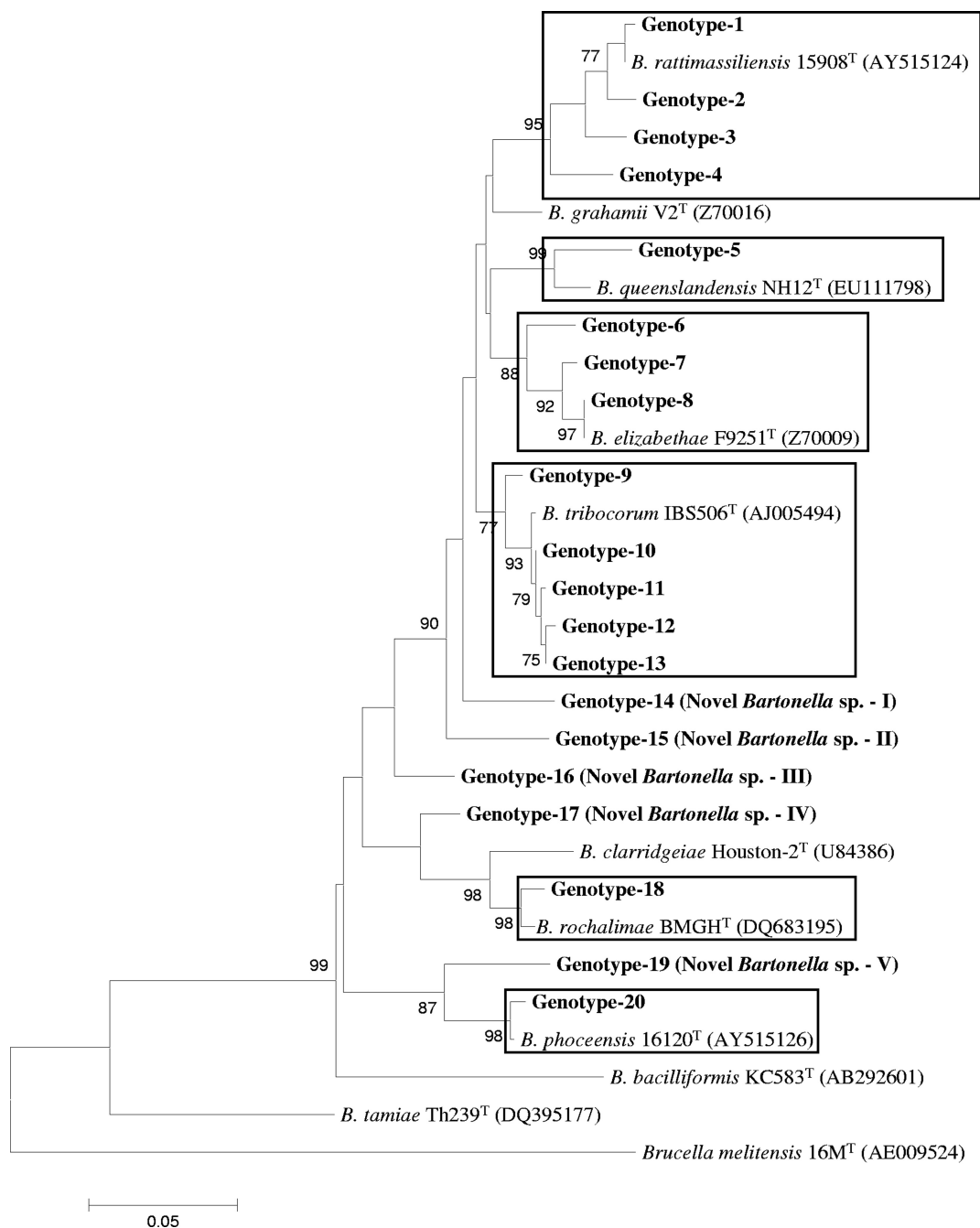


FIG. 2. Phylogenetic classification of *Bartonella* genotypes found in small mammals of Nepal based on *gltA* gene sequences. The phylogram was constructed by the neighbor-joining method with the Kimura 2-parameter model. Only the bootstrap values above 70% obtained from 1,000 replicates are given. The 37 different sequences of *gltA* from small mammals of Nepal were classified into seven clusters. The GenBank accession numbers for reference sequences are given in parentheses. Only known reference *Bartonella* sequences without gaps and missing data are included in the phylogram. The *gltA* gene sequence of *Brucella melitensis* 16M^T was included as an out-group.

where they can come into close contact with humans, who might therefore be at some risk of exposure.

In this study, specificity of some bartonellae for host animals was observed (Table 3). The *R. rattus brunneusculus* rats carried almost all the rat-origin *Bartonella* species detected in this study, except *B. rochalimae*, which was isolated recently from

R. norvegicus in Taiwan (19). In our study, *S. murinus* shrews harbored mostly *B. queenslandensis*. Studies by Bai et al. conducted in Dhaka, Bangladesh (3), also yielded evidence of the host specificity of *B. queenslandensis* for *S. murinus*. In addition, *S. murinus* carried some novel bartonellae in our study. On the other hand, *S. murinus* did not harbor other bartonell-

TABLE 3. *Bartonella* coinfections in Nepalese small mammals

Host species	No. of animals	District	Area ^a	Cosurviving <i>Bartonella</i> combination	Genotype(s) for:	
					<i>rpoB</i>	<i>gltA</i>
<i>Bandicota bengalensis</i>	1	Kathmandu	Kalimati	<i>B. cooperplainsensis</i> and <i>B. queenslandensis</i>	35, 11	
<i>Rattus rattus brunneusculus</i>	1	Lalitpur	NK	<i>B. cooperplainsensis</i> and <i>B. phoceensis</i>	33, 27	20
	1	Lalitpur	NK	Novel <i>Bartonella</i> sp. and <i>B. rattimassiliensis</i>	29, 30, 31	1, 2
	1	Lalitpur	NK	<i>B. cooperplainsensis</i> and <i>B. rattimassiliensis</i>	34	3
	1	Lalitpur	NK	<i>B. elizabethae</i> and <i>B. tribocorum</i>	24	9
<i>Suncus murinus</i>	10	Kathmandu	Hyumata	<i>B. queenslandensis</i> and novel <i>Bartonella</i> sp.	13	14
		Lalitpur	Patan		1	15, 17
		Kathmandu	Sabalbahal		10	17
		Kathmandu	Sabalbahal		13	14
		Kathmandu	NK		15, 36	17
		Kathmandu	NK		1	16
		Kathmandu	NK		2	15
		Kathmandu	NK		2	15
		Kathmandu	NK		13	14
		Lalitpur	NK		16	17
	1	Kathmandu	Sabalbahal	<i>B. queenslandensis</i> and <i>B. rochalimae</i>	13	18
1	Lalitpur	NK	<i>B. elizabethae</i> and novel <i>Bartonella</i> sp.	23, 36		
Total	17					

^a NK, not known.

lae, such as *B. cooperplainsensis*, *B. phoceensis*, and *B. rattimassiliensis*. These findings appear to suggest specificity of the bacteria for the host.

The prevalence and diversity of *Bartonella* species in animals or other hosts are controlled by geographical location, environmental conditions, the presence of vectors, and host species and habitats. In our study, 17 animals (15.7%) were infected with multiple *Bartonella* species and most of these animals had dual infections with different combinations of species (Table 4). This may be due to vector transmission between different animal hosts. According to Ellis and others, *Bartonella* species associated with hosts native to the Old World are phylogenetically distinct from those associated with hosts native to the New World (10). In this study, however, genotypes belonging to *B. elizabethae*, *B. phoceensis*, *B. rattimassiliensis*, and *B. tribocorum* were closely related to the isolates found in America and Europe (Tables

5 and 6), suggesting that carriers of these species might have migrated from Asia to other continents. Moreover, some novel genomic groups detected in this study were 98.3 to 100% identical to *Bartonella* strains found in Africa (Gundi et al., unpublished data) (Table 5). Furthermore, some genotypes, including novel genomic groups, from Nepalese rodents and shrews clustered well with other well-known rodent-associated species and human pathogens, such as *B. cooperplainsensis*, *B. elizabethae*, *B. phoceensis*, *B. queenslandensis*, *B. rattimassiliensis*, *B. rochalimae*, and *B. tribocorum* (Fig. 1 and 2). These findings suggest that small mammals in Nepal, like those in other Asian countries, might be a potential source of *Bartonella* infections. Furthermore, these results suggest the need to conduct further studies to verify whether these agents might be responsible for human cases of febrile illness of unknown etiology and to determine the evolu-

TABLE 4. Association between *Bartonella* genotypes and mammalian hosts

<i>Bartonella</i> species	No. of animals associated with specific <i>Bartonella</i> organism(s)				Total
	<i>Bandicota bengalensis</i>	<i>Rattus rattus brunneusculus</i>	<i>Suncus murinus</i>	<i>Mus musculus castaneus</i>	
<i>B. cooperplainsensis</i>	1	3			4
<i>B. elizabethae</i>		5	1		6
<i>B. phoceensis</i>		1			1
<i>B. rattimassiliensis</i>		3			3
<i>B. rochalimae</i>	1		1		2
<i>B. tribocorum</i>	1	4	2		7
<i>B. queenslandensis</i>	1	2	47		50
Novel <i>Bartonella</i> sp.		3	12		15
Combination of species	1	4	10		15
Total no. of associated animals/total no. of animals	5/38	25/90	73/92	0/3	103/223

TABLE 5. Genetic relationships between *Bartonella* species detected in Nepal and those from other geographical regions based on *mpoB* sequence analysis

<i>Bartonella</i> sp. identified	Genotype(s) determined in this study	Host animal(s) in this study ^a	Accession no. of related GenBank sequence	% Sequence similarity	Host(s) of related sequence	Country of host(s)	Reference ^b	
<i>B. coopersplainsensis</i>	32	RRB	EU714973	100	<i>Rattus rattus</i>	Laos	1	
			EU111792	100	<i>Rattus leucopus</i>	Australia	13	
<i>B. phoceensis</i>	27	RRB	AY515132	99.5	<i>Rattus norvegicus</i>	France	12	
			AB290278	99.5	<i>Rattus rattus</i>	Japan	16	
Novel <i>Bartonella</i> sp.	28	RRB	FJ667575	98.8	Unknown	Taiwan	D. D. Ji et al., unpublished data	
<i>B. queenslandensis</i>	1–19	SM	EF204538	98.1–99.3	<i>Suncus murinus</i>	Bangladesh	3	
			EF204539	97.2–100				3
			AB290268	97.2–100	Unknown	Japan	16	
			AB290269	97.1–99.9	Unknown			
			AB290270	97.6–98.8	<i>Rattus rattus</i>	Japan	16	
			AB290266	100	<i>Rattus rattus</i>	Australia	13	
			EU111787	98.4	<i>Melomys</i> sp.			
			EU111788	99.2	<i>Rattus tunneyi</i>			
			EU111789	98.8	<i>Rattus fuscipes</i>			
			EU111790	99.5	<i>Rattus conatus</i>			
EU111791	99.3	<i>Rattus leucopus</i>						
Novel <i>Bartonella</i> sp.	23	SM	FJ851123	98.3	<i>Lophuromys rita</i>	Democratic Republic of Congo	Gundi et al., unpublished	
<i>B. tribocorum</i>	22	BB, RRB	FJ851124	100	<i>Mus minutoides</i>			
			FJ851125	100	<i>Mus minutoides</i>			
			FJ851126	100	<i>Mus minutoides</i>			
			FJ851129	100	<i>Mus minutoides</i>			
			FJ851144	98.3	<i>Lophuromys</i> sp. (<i>Lophuromys dudui</i> related)	Tanzania	Gundi et al., unpublished	
			FJ851146	98.2	<i>Lophuromys</i> sp. (<i>Lophuromys dudui</i> related)			
<i>B. tribocorum</i>	22	BB, RRB	EU714974	99.9	<i>Rattus rattus</i> , <i>Mus cervicolor</i> , <i>Canomys badius</i> , <i>Rattus exulans</i>	Laos	1	
			EU714975	99	Unknown			
<i>B. elizabethae</i>	24	RRB	AF165992	98.3	Unknown	France	23	
			FJ851128	98.1	<i>Arvicanthus neumanni</i>	Democratic Republic of Congo	Gundi et al., unpublished	
<i>B. rochalimae</i>	37	BB	EU551156	99.6	<i>Rattus norvegicus</i>	Taiwan	19	

^a BB, *Bandicota bengalensis*; RRB, *Rattus rattus brunneusculus*; SM, *Suncus murinus*.^b The unpublished data cited are found in the GenBank database.

TABLE 6. Genetic relationships between *Bartonella* species detected in Nepal and those from other geographical regions based on *gltA* sequence analysis

<i>Bartonella</i> sp. identified	Genotype determined in this study	Host animal(s) in this study ^a	Accession no. of related GenBank sequence	% Sequence similarity	Host of related sequence	Country of host	Reference ^b
<i>B. rattimassiliensis</i>	1	RRB	FJ179375	100	<i>Rattus norvegicus</i>	Taiwan	19
			AY515125	100	<i>Rattus norvegicus</i>	France	12
	3	RRB	AB290283	100	<i>Rattus rattus</i>	Japan	16
			AF342933	100	<i>Rattus fuscipes</i>	China	28
			FJ464242	100	<i>Rattus</i> sp.	China	D. Li et al., unpublished data
			FJ464244	100	<i>Rattus</i> sp.		
			FJ492787	100	<i>Rattus tanezumi</i>		
			FJ492792	100	<i>Rattus tanezumi</i>		
		FJ589047	100	<i>Rattus tanezumi</i>			
<i>B. elizabethae</i>	7	RRB	AY589561	100	<i>Bandicota bengalensis</i>	Bangladesh	3
	8	RRB	AB445000	100	<i>Acomys cahirinus</i>	Japan	15
			DQ884386	100	<i>Rattus norvegicus</i>	China	Li et al., unpublished
			DQ884390	100	<i>Rattus norvegicus</i>	China	Li et al., unpublished
			FJ655404	100	<i>Rattus exulans</i>	Thailand	2
			FJ946849	100	Dog	Thailand	Y. Bai et al., unpublished data
			U28072	100	Unknown	Unknown	D. C. Jones et al., unpublished data
			Z70009	100	Human	United States	9
			Z70020	100	<i>Rattus</i> sp.	Peru	5
	6	RRB	AF329679	100	<i>Rattus fuscipes</i>	China	28
			FJ179377	100		Taiwan	J. W. Hsieh et al., unpublished data
			FJ179391	100		Taiwan	Hsieh et al., unpublished
			FJ492786	100	<i>Rattus tanezumi</i>	China	Li and Liu, unpublished
			FJ492791	100	<i>Rattus tanezumi</i>		
			FJ492793	100	<i>Rattus tanezumi</i>		
			FJ492797	100	<i>Rattus tanezumi</i>		
			FJ589045	100	<i>Rattus tanezumi</i>	China	Li et al., unpublished
			FJ589046	100	<i>Rattus tanezumi</i>		
		FJ589050	100	<i>Rattus tanezumi</i>			
		FJ589052	100	<i>Rattus tanezumi</i>			
		FJ589053	100	<i>Rattus tanezumi</i>			
		FJ589058	100	<i>Rattus tanezumi</i>			
		FJ589061	100	<i>Rattus tanezumi</i>			
		FJ589062	100	<i>Rattus tanezumi</i>			
<i>B. queenslandensis</i>	5	RRB	AB290280	100	<i>Rattus rattus</i>	Japan	16
			AY589566	100	<i>Rattus rattus</i>	Bangladesh	3
			FJ179376	100		Taiwan	19
			FJ179384	100			
			FJ946846	100	Dog	Thailand	Bai et al., unpublished
<i>B. tribocorum</i>	13	RRB, BB	AF086636	100	<i>Rattus rattus</i>	Portugal	10
	10	RRB, BB	AF075164	100	<i>Rattus norvegicus</i>	United States	10
			AJ583111	100	Unknown	South Africa	22
			AY902183	100	<i>Rattus tanezumi</i>	Indonesia	27
			AY902188	100			
			AY902189	100			
			AY902191	100			
			DQ884383	100	<i>Rattus norvegicus</i>	China	Li et al., unpublished
			DQ884387	100			
			DQ884388	100			
			DQ884389	100			
			DQ884391	100			
			DQ884392	100			
			EF051466	100	<i>Rattus norvegicus</i>	China	Li et al., unpublished
			FJ464203	100	<i>Rattus norvegicus</i>	China	Li et al., unpublished
			FJ464205	100			
		FJ464206	100				

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TABLE 6—Continued

<i>Bartonella</i> sp. identified	Genotype determined in this study	Host animal(s) in this study ^a	Accession no. of related GenBank sequence	% Sequence similarity	Host of related sequence	Country of host	Reference ^b
			FJ492782	100	<i>Rattus tanezumi</i>	China	Li and Liu, unpublished
			FJ492783	100	<i>Rattus tanezumi</i>		
			FJ492785	100	<i>Rattus tanezumi</i>		
			FJ492789	100	<i>Rattus tanezumi</i>		
			FJ492794	100	<i>Rattus tanezumi</i>		
			FJ492795	100	<i>Rattus tanezumi</i>		
			FJ492798	100	<i>Rattus tanezumi</i>		
			FJ492800	100	<i>Rattus tanezumi</i>		
			FJ589051	100	<i>Rattus tanezumi</i>	China	Li et al., unpublished
			FJ589057	100	<i>Rattus tanezumi</i>		
<i>B. rochalimae</i>	18	SM	EU551154	100	<i>Rattus norvegicus</i>	Taiwan	19

^a BB, *Bandicota bengalensis*; RRB, *Rattus rattus brunneusculus*; SM, *Suncus murinus*.

^b The unpublished data cited are found in the GenBank database.

tionary, genetic, and pathogenic relationships among Nepal isolates and other isolates in Asia and on other continents.

Nucleotide sequence accession numbers. The DNA sequences obtained in this study were deposited in GenBank under accession numbers GU143433 to GU143508 (for *rpoB*) and GU143509 to GU143549 (for *gltA*) (see Tables S1 and S2 in the supplemental material).

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