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NOTE

The Presence of *Borrelia valaisiana*-Related Genospecies in Ticks and a Rodent in Taiwan

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A field survey was conducted to investigate the presence of *Borrelia burgdorferi sensu lato* (*s.l.*) in six counties of Taiwan. Spirochetes were successfully isolated from one rodent ear sample out of 485 rodent ears and 53 live, fed tick (*Ixodes granulatus*) samples. The spirochetes were confirmed to be *B. burgdorferi s.l.* by real-time PCR. In addition, 23 of 113 tick samples were tested positive for *Borrelia* DNA according to real-time PCR. The *Borrelia* isolate from the rodent and the 23 *Borrelia* DNA samples from the ticks were identified as *B. valaisiana*-related genospecies by phylogenetic analysis based on flagellin gene sequences. These findings suggest that the *Borrelia valaisiana*-related strains are maintained in a zoonotic cycle between tick vectors and reservoir hosts in Taiwan.

***Keywords*:** *Borrelia valaisiana*-related, ticks, rodent

The spirochete group *Borrelia burgdorferi sensu lato* (*B. burgdorferi s.l.*) is composed of at least 13 species of tick-borne obligate parasites (Burgdorfer *et al.*, 1982; Wang *et al*., 1999; Masuzawa *et al*., 2001; Richter *et al*., 2006; Postic *et al*., 2007). The species depend on tick vectors and a wide range of vertebrate animals, including small mammals, lizards and birds, for their transmission (Anderson and Magnarelli, 1984; Anderson *et al*., 1986; Anderson, 1989; Clark *et al*., 2005). In 2008 and 2009, a field survey was conducted to investigate the presence of *B. burgdorferi s.l.* in ticks and rodents in six counties in Taiwan including three off-shore islands (Lienchiang, Kinmen, and Penghu counties) and three eastern counties (Yilan, Hualien, and Taitung counties). Ear samples from 485 captured rodents were cultured for *Borrelia* in Barbour-Stoenner-Kelly-H (BSK-H) media. After the rodents were euthanized with ether, ear samples from the rodents were sterilized with iodine and inoculated into BSK-H medium. A total of 113 fed ticks (*Ixodes granulatus*: 92 adults, 10 nymphs, and 11 larvae) were collected from the rodents, and 53 live ticks were surface sterilized with iodine and individually ground with microtube pestles in BSK-H medium. Half of each sample was added to BSK-H medium for culture. The cultures were incubated at 32°C and examined for spirochetes by dark field microscopy once per week for seven weeks. One spirochete-positive culture (designated 404 m) from a *Mus caroli* rodent captured in Taitung County was identified by dark field microscopy, but no spirochetes were identified from the tick samples.

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The DNA extracted from the spirochete isolate 404m was confirmed to be *B. burgdorferi s.l.* by real-time PCR of the *hbb* gene (Portnoi *et al*., 2006). Tick samples (53 lived and 60 dead ticks) were ground with microtube pestles in BSK-H medium as described above, and DNA was extracted from half of the sample using the QIAamp DNA Mini kit (QIAGEN) according to the manufacturer's instructions. The DNA from the ticks was used for *hbb* real-time PCR amplification to detect the presence of *Borrelia*. Tweenty-three (21 adults and 2 nymphs) out of 113 ticks were tested positive for *B. burgdorferi s.l.* The ticks that were tested positive were collected from Lienchiang, Kinmen, Hualien, and Taitung counties and had a total infection rate of 20.4%.

The *hbb* real-time PCR could identify *B. burgdorferi s.l.* genospecies in a single PCR run based on sequence polymerphisms within the *hbb* gene (Portnoi *et al.*, 2006). The real-time PCR samples were prepared using LC FastStart DNA Master^{plus} HybProbe in a LightCycler rapid thermal cycler system (Roche Diagnostics), and the amplification program was followed by a melting program, which started at 48°C for 30 sec and increased to 75°C at 0.1°C/sec, with continuous fluorescence monitoring. After real-time PCR amplification, a melting curve was generated for genotyping, yielded melting temperatures ranging from 58.62 to 60.27°C (Table 1). The melting temperatures of *B. valaisiana* (58.99±0.12°C) and *B. spielmanii* (59.08°C) were too close to allow clear differentiation (Portnoi *et al*., 2006). Phylogenetic relationships based on the alignment of partial sequences of the flagellin gene were investigated to clarify the identity of 24 real-time PCR samples positive for *B. burgdorferi s.l.* (Masuzawa *et al*., 2004).

Table 1. List of *B. valaisiana*-related strains from Taiwan identified in this study

Strain	Geographic origin	Source of strain isolation or detection	*hbb* Tm value
404m	Taitung County	*Mus caroli*	59.32
TKM-1	Kinmen County	*Ixodes granulatus* (adult female)	59.95
TKM-2	Kinmen County	*I. granulatus* (adult female)	60.27
TKM-3	Kinmen County	*I. granulatus* (adult female)	60.13
TKM-4	Kinmen County	*I. granulatus* (adult female)	60.17
TKM-6	Kinmen County	*I. granulatus* (adult female)	59.99
TKM-7	Kinmen County	*I. granulatus* (adult female)	59.88
TKM-14	Kinmen County	*I. granulatus* (adult female)	59.61
TKM-29	Kinmen County	*I. granulatus* (adult female)	59.19
TKM-30	Kinmen County	*I. granulatus* (adult female)	58.62
TKM-36	Kinmen County	*I. granulatus* (adult female)	59.78
TKM-40	Kinmen County	*I. granulatus* (adult female)	59.79
TLC-22	Lienchiang County	*I. granulatus* (adult female)	60.01
TLC-26	Lienchiang County	*I. granulatus* (nymph)	59.84
TLC-27	Lienchiang County	*I. granulatus* (nymph)	59.80
TLC-42	Lienchiang County	*I. granulatus* (adult female)	60.01
THL-6	Hualien County	*I. granulatus* (adult female)	60.20
THL-7	Hualien County	*I. granulatus* (adult female)	60.00
THL-8	Hualien County	*I. granulatus* (adult female)	59.96
THL-10	Hualien County	*I. granulatus* (adult female)	60.02
THL-11	Hualien County	*I. granulatus* (adult female)	59.97
THL-14	Hualien County	*I. granulatus* (adult female)	60.15
TTT-1	Taitung County	*I. granulatus* (adult female)	60.10
TTT-3	Taitung County	*I. granulatus* (adult female)	60.25
*35210	USA	*I. dammini*	68.45
*51991	Japan	*I. persulatus*	51.65
*51567	Switzerland	*I. ricinus*	65.6

* ATCC 35210 (*B. burgdorferi* B31), ATCC 51991 (*B. garinii* Fuji P1), and ATCC 51567 (*B.afzelii* CIP) were used as reference strains to generate the melting curve.

Corresponding sequences of 16 control strains from GenBank were used for comparison. In a phylogenetic tree constructed by the neighbor-joining method, all of the strains in this study formed a separate clade and were easily distinguished from *B. spielmanii*, which was not identified in the *hbb* melting curve experiments (Fig. 1). Within the same clade, these 24 strains clustered closely with previously reported *B. valaisiana*-related strains from northeast Asia (10MT), southwest China (QLZSP1 and QSYSP3) and Taiwan (KR1, KR3, TM1, and TA1) (Masuzawa *et al*., 2000, 2004; Chu *et al*., 2008b). The phylogenetic tree supports the separation of *B. valaisiana*-related strains and the *B. valaisiana* group with a bootstrap value of 98%. These results reveal that all of the strains detected in the ticks as well as the strain isolated from the rodent in Taiwan belong to the *B. valaisiana*-related group and are distinct from the European *B. valaisiana* group.

In conclusion, *Borrelia* strains obtained in Taiwan appear to be genetically similar to the *B. valaisiana*-related group found in eastern Asia and southwestern and southeastern China (Masuzawa *et al*., 2004; Chu *et al*., 2008a, 2008b; Chao *et al*., 2010). We provide the first evidence of *B. valaisiana*-related genospecies in vector ticks and a rodent host, suggesting that *B. valaisiana*-related strains circulate between rodent reservoir hosts and tick vectors in Taiwan. This is different from the transmission cycle of *B. valaisiana* in Europe, where *B.* valaisiana has been found in various avian reservoirs (Kurtenbach *et al*., 1998; Hanincova *et al*., 2003). The pathogenic abilities of *B. valaisiana*-related strains are still unclear, and it remains to be determined whether this *B. burgdorferi s.l.* species can cause disease in humans.

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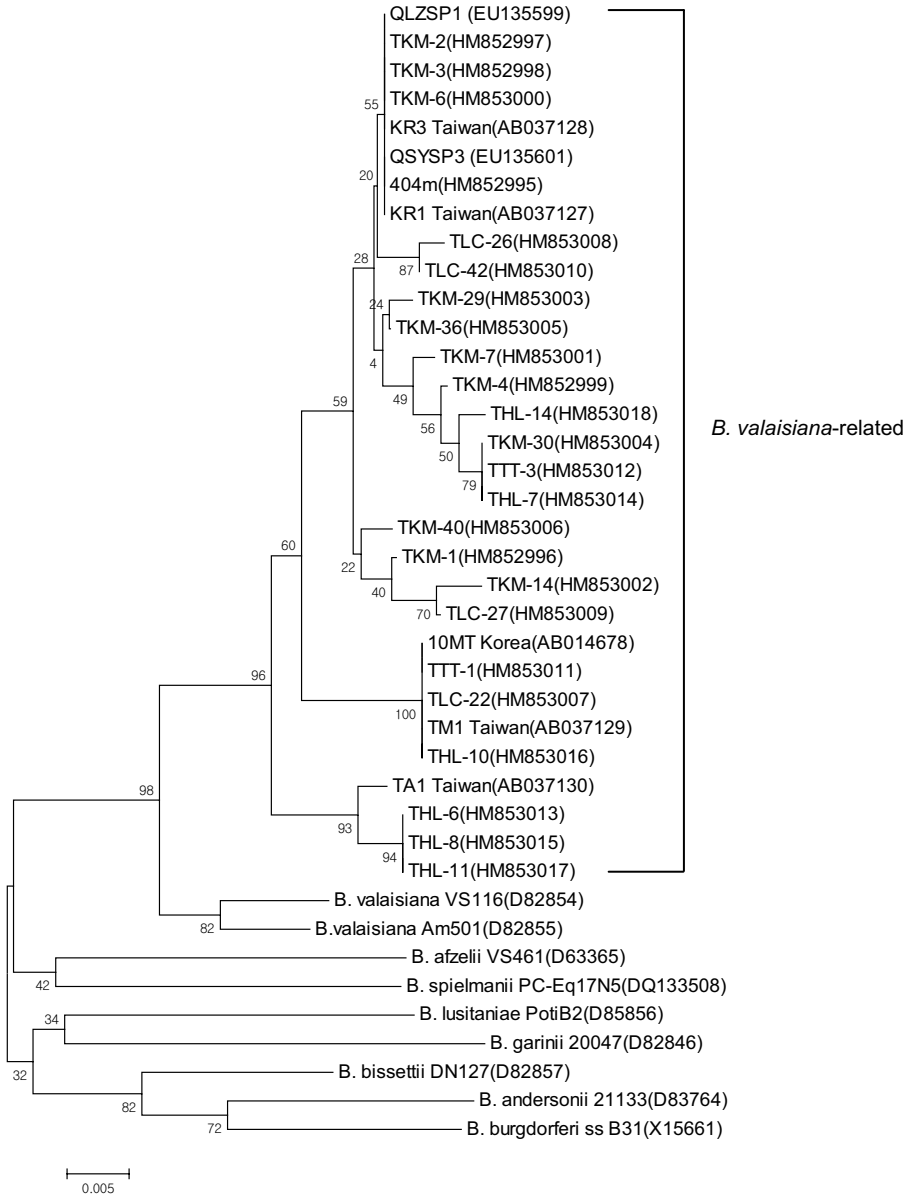

Fig. 1. Phylogenetic tree based on a comparison of flagellin gene sequences from 24 Taiwan strains identified in this study and 16 reference strains. The tree was made with *MEGA* version 4 software. Accession numbers of each strain are indicated in parentheses.

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