10.1111/j.1469-0691.2008.02183.x

Comparison of the performances of MLVA vs. the main other typing techniques for *Bartonella henselae*

R. Bouchouicha[1], H.-J. Boulouis[1], M. Berrich[1], M. Monteil[1], B. Chomel[2] and N. Haddad[1]

[1]UMR BIPAR, ENVA/AFSSA/INRA, Maisons-Alfort, France and [2]School of Veterinary Medicine, University of California, Davis, CA, USA

INTRODUCTION

Bartonella henselae is a zoonotic pathogen of growing medical importance, which causes persistant bacteraemia in the feline reservoir host. Transmission to the incidental human host, which occurs essentially by cat scratch, is associated with most human cases of cat scratch disease but also with bacillary angiomatosis and peliosis, and other clinical manifestations (endocarditis, osteomyelitis, neuroretinitis…).

Two different 'genotypes' (I/II) were described in 1996, based on minor sequence differences in the 16S rDNA.

Different molecular techniques have been developed for *B. henselae* typing, revealing high genetic diversity among isolates. The most widely used has been pulsed field gel electrophoresis (PFGE). Recently, two other techniques have been developed: multilocus sequence typing (MLST) in 2003 and mutispacer typing (MST) in 2006, both based on the sequencing of small genomic sequences (nine different segments each). With MLST, Iredell *et al.* (2003) obtained only seven profiles for 37 tested isolates. Using MST, Li *et al.* (2006) detected 39 profiles for 126 cat isolates and 16 profiles for 75 human isolates.

MLVA (multiple locus VNTR analysis) was developed in 2007 in our laboratory, based on the polymorphism of sequences called VNTRs (variable number tandem repeats). This technique involved the amplification of five main VNTRs, called BHV-A to E (for *Bartonella henselae* VNTRs). Thirty-one profiles were observed for 43 tested isolates [6].

In the present study, we compared MLVA performances with those of PFGE, MLST and MST, using common isolates and strains that had been previously tested by at least one of these techniques.

Corresponding author and reprint requests: Nadia Haddad, UMR BIPAR, ENVA/AFSSA/INRA, Maisons-Alfort, France.
E-mail: nhaddad@vet-alfort.fr

No conflicts of interest declared.

MATERIALS AND METHODS

For comparing our data with those provided by other techniques, we used five bibliographic sources [1–5]. In these studies, at least one part of the tested isolates was the same as that tested by MLVA (17, 22 and 65 common isolates with MST, MLST and PFGE, respectively). PFGE was used in four studies, [1–4] and 22, 17, 21 and 5 isolates, respectively, were common to those tested using MLVA. We evaluated the profile numbers obtained for the same series of isolates for each technique and we calculated the global diversity index proposed by Hunter and Gaston.

RESULTS

The performances (profile numbers and diversity indexes, DIs) of the different typing techniques are presented in Table 1. Both profile numbers, numbers of unique profiles and DIs were almost systematically higher for MLVA, for the same series of isolates.

MLVA-MLST

For MLST and MLVA, the profile numbers were, respectively, 6/22 and 14/22, which corresponded to DIs of 0.61 and 0.89, respectively. Both MLVA and MLST distinguished without ambiguity three unique profiles corresponding to three strains. Among the 13 isolates (60%) found identical using MLST (isolates belonging to Sequence Type (ST) '1'), MLVA differentiated easily seven different profiles. For MLVA, there were 11 unique profiles vs. only three for MLST. In addition, MLVA allowed distinguishing some isolates within two additional groups of isolates found identical by MLST (ST '4'and '5').

MLVA-MST

Data comparison reflects the high discriminatory powers of both techniques. The profile numbers

Table 1. Comparison of the results obtained by MLVA vs. the other typing techniques: diversity indexes and profile numbers

Authors (references)	Common isolates	Geographic origins	16 S RNA genotype		Typing technique (vs. MLVA)	Number of profiles		Diversity index (D.I.)		No of unique profiles		No.of clusters
			Genotype I	Genotype II		MLVA	Other	MLVA	Other	MLVA	Other	
Arvand *et al.*, 2001[1]	22	Germany (21), USA (1)	3	19	PFGE	11	7	0.83	0.75	7	6	ND
Chang *et al.*, 2002 [2]	17	USA	2	15	PFGE	<6	7	0.74	0.87	2	4	ND
Iredell *et al.*, 2003 [3]	20	Australia (20), Germany (1), USA (1)	13	7	PFGE	12	6	0.85	0.82	9	1	3
Maruyama *et al.*, 2001 [4]	5	USA (4), Japan (1)	3	2	PFGE	5	3	0.75	0.6	5	2	ND
Iredell *et al.*, 2003 [3]	22	Australia (20), Germany (1), USA (1)	14	8	MLST	14	6	0.89	0.61	11	3	3
Li *et al.*, 2006 [5]	17	USA (10), Philippines (7)	13	4	MST	17	12	1	0.94	17	9	4

ND = Not determined.

and DIs were 12/17 and 0.94 for MST and 17/17 and 1 for MLVA. MST allowed obtaining only 9/17 (53%) unique profiles instead of 17/17 (100%) for MLVA. The different isolates found identical by MST (types '5', '18' and '38') were differentiated by MLVA. For both techniques, no profile was common to genotypes I and II.

MLVA-PFGE

The comparison between MLVA and PFGE was based on 22, 17, 20 and 5 isolates in common [1–4]. For defining isolates as belonging to two different PFGE types, we considered as a minimum the presence of a two-band difference, as the criteria applied by the different authors were not the same. This option was strengthened by the observation that in most cases isolates that differed by only one band had identical profiles by MLVA technique. As shown in Table 1, apart from in one study [2], MLVA revealed more polymorphism than PFGE. The same clusters were defined when PFGE [2] and MLVA were applied on groups of isolates from owners and their cats, demonstrating the reliability of MLVA for molecular epidemiological studies. Some cases of discrepancies between genotypes I/II and PFGE types were found [1,4], whereas no such divergences were observed with MLVA, MLST and MST.

CONCLUSIONS

The techniques developed before 2007 have been very useful for epidemiological studies on *B. henselae*. MLST and MST are easy to use and easy to analyse and interpret, as they provide simple numerical data. But PFGE is based on complex banding patterns and there is no clear consensus for determining the number of bands that allow distinguishing two PFGE types. MLVA appears the most interesting: it is simple, not expensive, does not need sequencing like MLST and MST and has the highest discriminatory power in terms of profile numbers and DIs.

REFERENCES

1. Arvand M, Klose AJ, Schwartz-Porsche D, Hahn H, Wendt C. Genetic variability and prevalence of *Bartonella henselae* in cats in Berlin, Germany, and analysis of its genetic relatedness to a strain from Berlin that is pathogenic for humans. *J Clin Microbiol* 2001; **39:** 743–746.
2. Chang CC, Chomel BB, Kasten RW, Tappero JW, Sanchez MA, Koehler JE. Molecular epidemiology of *Bartonella henselae* infection in human immunodeficiency virus-infected patients and their cat contacts, using pulsed-field gel electrophoresis and genotyping. *J Infect Dis* 2002; **186:** 1733–1739.
3. Iredell J, Blanckenberg D, Arvand M, Grauling S, Feil EJ, Birtles RJ. Characterization of the natural population of *Bartonella henselae* by multilocus sequence typing. *J Clin Microbiol* 2003; **41:** 5071–5079.
4. Maruyama S, Sakai T, Morita Y *et al.* Prevalence of *Bartonella* species and 16S rRNA gene types of *Bartonella henselae* from domestic cats in Thailand. *Am J Trop Med Hyg* 2001; **65:** 783–787.
5. Li W, Chomel BB, Maruyama S *et al.* Multispacer typing to study the genotypic distribution of *Bartonella henselae* populations. *J Clin Microbiol* 2006; **44:** 2499–2506.
6. Monteil M, Durand B, Bouchouicha R *et al.* Development of discriminatory multiple-locus variable-number tandem repeat analysis for *Bartonella henselae*, *Microbiology* 2007; **153:** 1141–1148.