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Case Report

Molecular characterization of *Leptospira sp* by multilocus variable number tandem repeat analysis (MLVA) from clinical samples: a case report

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SUMMARY

Leptospirosis is a zoonotic infection for which diagnosis is difficult. It has appeared as a global emerging infectious disease over recent years. Genotype determination often requires a *Leptospira* strain obtained by culture, which is a long and fastidious technique. A method based on multilocus variable number tandem repeat analysis (MLVA) to determine the genotype of *Leptospira interrogans*, performed directly on blood or urine samples, is proposed. This method was applied to a fatal case of leptospirosis for which the geographical origin of infection was unknown. This technique will allow a genotype to be obtained for *L. interrogans*, even when cultures remain negative.
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1. Introduction

Leptospirosis is a zoonotic infection for which diagnosis is difficult. The genus *Leptospira* includes both saprophytic and pathogenic species, with more than 300 serovars described. Serovars are useful for epidemiological studies and can be related to a particular reservoir host and/or geographic area. These serovars can be determined by serological typing, or can be deduced from genotypes, which are characterized by molecular typing methods such as variable number tandem repeat (VNTR) analysis, also called multilocus VNTR analysis (MLVA) when many loci are studied.[1–3] However, determination of the genotype, even by molecular techniques, often requires a *Leptospira* strain obtained by culture, which is a long (more than 3 weeks), fastidious, and highly uncertain technique.

The aim of this study was to determine the genotype of a *Leptospira interrogans* strain by MLVA, directly on blood and urine samples, in a fatal and complicated case of leptospirosis.

2. Case report

A 60-year-old man presented to the university hospital with fever, 18 days after returning from a 10-day trip to Thailand. During his trip, the patient had taken no prophylaxis against malaria, had taken part in unprotected intercourse, and had bathed in the swimming pool of his vacation club. One week before the onset of symptoms, the patient went swimming in the river of his garden in mainland France. He had a previous history of arterial hypertension and restless leg syndrome.

On admission, the patient presented fever and myalgia (which had been present for 3 days), vomiting, and pollakiuria, and a physical examination revealed plantar pruritis. Laboratory results showed thrombocytopenia (platelets 132 $\times$ 10^9/l), polynucleosis (neutrophils 10.62 $\times$ 10^9/l), a raised C-reactive protein (CRP; 203.2 mg/l), and acute renal insufficiency (estimated glomerular filtration rate (GFR) 15 ml/min/1.73 m^2). Tests for malaria, hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, and arboviruses were all negative. A cyto-bacteriological examination of urine showed leukocyturia (600 leukocytes/mm^3). Blood and urine cultures remained negative. On day 2, an acute respiratory distress syndrome was evident, and the renal insufficiency

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increased. There were clinical and radiological signs of pulmonary oedema. Treatment with diuretics and ceftriaxone was initiated. The patient was then transferred to an intensive care unit and was intubated. At the end of the day 2, the patient's situation worsened with the appearance of major hepatic cytolysis. Finally, the patient developed heart failure and suffered refractory shock complicated by a fatal cardiac arrest. The diagnosis of leptospirosis was made post-mortem from urine and blood samples by real-time PCR, as described previously.[4]

3. Methods and results

Identification of the *L. interrogans* species was confirmed by 16S rDNA sequencing performed on blood samples. MLVA was then performed on VNTR-4, VNTR-7, VNTR-10, and VNTR-19 loci using the blood and urine specimens and an original PCR method based on 90 cycles of amplification. DNA was extracted using an Invisorb Spin DNA Extraction Kit (STRATEC Molecular, Berlin, Germany), according to the manufacturer's instructions. DNA was then amplified using HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany) with one denaturation cycle at 94 °C for 10 min; 90 cycles of denaturation at 94 °C for 10 s, annealing at 56 °C for 30 s, and elongation at 72 °C for 1 min; and a final elongation at 72 °C for 10 min. The primers used for analysis of VNTR-4 were VNTR-4a (5′-AAGTAAAAGCGCTCCCAAGA-3′) and VNTR-4b (5′-ATAAAGGAAGCTCGGCGTTT-3′);[3] for VNTR-7 were VNTR-7a (5′-TCATCTGCTCCGGAGATTCG-3′) and VNTR-7b (5′-TCCCTCCACAGGTTGTCTTG-3′); for VNTR-10 were VNTR-10a (5′-TCCAAAATTCAGCCCTCAAG-3′) and VNTR-10b (5′-GACG CTTGGCATTTGTATCC-3′); and for VNTR-19 were VNTR-19a (5′- CAGAAACAAGAGGGAAGATTC-3′) and VNTR-19b (5′-ACTCT-CATTTAAGAGTGGCTG-3′).[1] The amplified products were analyzed by 1.5% agarose gel electrophoresis. The sizes of the amplified products were analyzed by comparison with a 100-bp ladder (New England BioLabs, Ipswich, USA), and the number of repeats (n) for each VNTR was then calculated with the size of the amplified products[1,3] (Figure 1). The formula used for VNTR-4 was 425 + 34n bp; for VNTR-7 was 166 + 46n bp; for VNTR-10 was 149 + 45n bp; and for VNTR-19 was 139 + 47n bp. Thus, two repeats were found for the VNTR-4 locus (size of amplicon 493 bp), one repeat for the VNTR-7 locus (size of amplicon 212 bp), one repeat for the VNTR-10 locus (size of amplicon 194 bp), and two repeats for the VNTR-19 locus (size of amplicon 233 bp). The *L. interrogans* typing patterns were the same for DNA isolated from urine and blood samples.

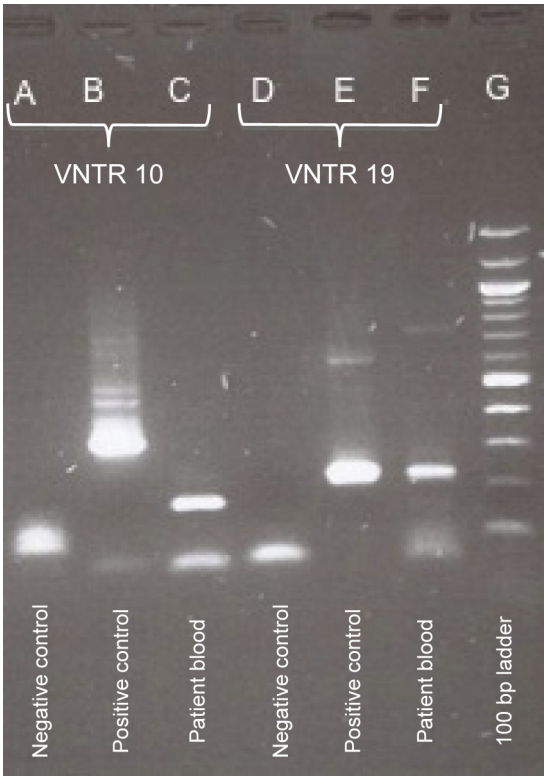

Figure 1. VNTR locus 10 and 19 size determination by agarose gel electrophoresis of amplified products. The VNTR loci evaluated were VNTR 10 (lanes A, B, and C) and VNTR 19 (lanes D, E, and F). Lanes A and D correspond to the negative control, lanes B and E correspond to an *L. interrogans* serogroup icterohaemorrhagiae serovar Copenhageni, and lanes C and F correspond to the DNA extracted from the blood sample of the patient. Lane G: 100-bp ladder.

4. Discussion

The MLVA type obtained for these loci on *L. interrogans* DNA was not an exact match for the serovar profiles described previously.[1–3] This genotype could correspond to a serovar that has not yet been characterized by MLVA, and this indicates that the infection could have occurred in Thailand rather than in mainland France based on the serological and genotyping information of the French National Reference Centre for leptospirosis.[1,3,4] Indeed, this could illustrate the diversity of *Leptospira sp* strains in tropical climates and could indicate that circulating strains in these endemic countries remain to be characterized. This method, which does not require strain culture, could therefore be used to improve epidemiological knowledge of *Leptospira sp*.

In the case presented, the diagnosis of leptospirosis was made post-mortem and the *Leptospira* strain was not obtained by culture. In such cases, when a culture cannot be performed or remains negative, discriminating strains by genotyping is difficult because of the need for *Leptospira* grown in culture for many genotyping methods. This case report illustrates the possibility of discriminating strains with VNTR analysis by increasing the number of PCR cycles in such situations. This should encourage the development of a database of MLVA profiles for *L. interrogans* serovars.

A disadvantage of VNTR analysis is that this method has been developed mainly on *L. interrogans* species and only a few studies have performed VNTR analysis on other *Leptospira* species such as *Leptospira santarosai*.[5] Thus, VNTR analysis still needs improvement by performing the technique on *L. interrogans* strains, but also on other *Leptospira* species. Moreover, it is also possible that in certain cases this approach will lack sensitivity, for example when the DNA load is very low or when there is a delay in sampling following the onset of disease.

Leptospirosis is a severe disease, with a high mortality rate. Serovar or genotype determination is essential to study the epidemiology of this emerging infectious disease. Culture is important to identify new circulating strains of *Leptospira*, but this technique is fastidious and quite long and can often remain negative. In this case, another diagnostic tool is necessary. Multilocus sequence typing performed directly on blood samples has been described by some authors, but requires a sequencing step and presents some limitations.[6] Another recent study has reported a nested PCR technique on samples with a low bacterial load for VNTR analysis.[4] The MLVA method is easy to perform, and even if the accumulation of mutations due to polymerase errors during the 90 cycles is possible, this does not impact on the size of the amplification product and thus on the number of tandem repeats. With more complete data on MLVA profiles of *L. interrogans* serovars, the method described here may allow the determination of serovars even if *L. interrogans* is not obtained by culture. The diagnostic approach described here is rapid, simple,

and reliable, and can be recommended for the characterization of *Leptospira* strains.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Majed Z, Bellenger E, Postic D, Pourcel C, Baranton G, Picardeau M. Identification of variable-number tandem-repeat loci in *Leptospira interrogans* sensu stricto. *J Clin Microbiol* 2005;**43**:539–45.
2. Pavan ME, Cairó F, Pettinari MJ, Samartino L, Brihuega B. Genotyping of *Leptospira interrogans* strains from Argentina by multiple-locus variable-number tandem repeat analysis (MLVA). *Comp Immunol Microbiol Infect Dis* 2011;**34**:135–41.
3. Salaün L, Mérien F, Gurianova S, Baranton G, Picardeau M. Application of multilocus variable-number tandem-repeat analysis for molecular typing of the agent of leptospirosis. *J Clin Microbiol* 2006;**44**:3954–62.
4. Bourhy P, Herrmann Storck C, Theodose R, Olive C, Nicolas M, Hochedez P, et al. Serovar diversity of pathogenic *Leptospira* circulating in the French West Indies. *PLoS Negl Trop Dis* 2013;**7**:e2114.
5. Hamond C, Pinna M, Medeiros MA, Bourhy P, Lilenbaum W, Picardeau M. A multilocus variable number tandem repeat analysis assay provides high discrimination for genotyping *Leptospira santarosai* strains. *J Med Microbiol* 2015;**64**(Pt 5):507–12.
6. Agampodi SB, Moreno AC, Vinetz JM, Matthias MA. Utility and limitations of direct multi-locus sequence typing on qPCR-positive blood to determine infecting *Leptospira* strain. *Am J Trop Med Hyg* 2013;**88**:184–5.