

ABSTRACT

Molecular Methods for the Detection and Typing of *Leptospira* spp.

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Molecular methods, using the polymerase chain reaction (PCR), were evaluated to detect and type *Leptospira* species in clinical material. In addition, the potential of these techniques to determine the molecular epidemiology of *Leptospira* was assessed. Clinico-pathological features of eight patients with fatal leptospirosis were studied concomitantly with the demonstration of leptospiral DNA or leptospirae in postmortem specimens. Of these fatal cases, seven had associated significant end-organ damage characterized by jaundice, acute renal failure (ARF), and cardiopulmonary complications. Respiratory distress (ARDS) was noted in three patients. These complications were directly involved in mortality. Thrombocytopaenia was found in 50% of the patients. The major serogroups identified by serology were Autumnalis and Cynopteri, which were involved in four cases, respectively. Serovar *copenhageni* was isolated from the aqueous humour and cerebrospinal fluid of one patient.

Leptospiral DNA and/or leptospires were detected in postmortem specimens by PCR, immunofluorescence (IF) microscopy, or culture. PCR analysis was the most sensitive technique and confirmed multi-system distribution of leptospires in the body, including the brain and heart. This is the first report of leptospires being demonstrated in the brain of humans. An internal control was developed, which facilitated the identification of samples which were inhibitory to amplification by PCR.

The three PCR-based typing methods (PCR-RFLP, AP-PCR & LS-PCR) employed in this study were corroborative and allowed for discrimination among *Leptospira* species and serovars (even within the same genomic species). In addition, they confirmed the identity of recent isolates from clinical cases, and allowed accurate identification of unknown *Leptospira* strains to the serovar level, supporting the concept of the serovar as the basic taxonomic unit. These results suggest that PCR-based fingerprinting can be applied to the molecular epidemiology of *Leptospira* spp., as well as for the identification and confirmation of *Leptospira* isolates in clinical material.