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Characterisation of IS900 loci in *Mycobacterium avium subspecies paratuberculosis* and development of a rapid multiplex PCR typing system

Tim Bull, Ivo Pavlik, Fouad El-Zaatari, John Hermon-Taylor

Tim Bull, John Hermon-Taylor, St. George's Hospital Medical School, London SW17 ORE, United Kingdom

Ivo Pavlik, Veterinary Research Institute, Brno, Czech Republic

Fouad El-Zaatari, Baylor College of Medicine, Houston, TX, United States

Author contributions: All authors contributed equally to the work.

Correspondence to: Tim Bull, St. George's Hospital Medical School, London SW17 ORE, United Kingdom

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Abstract

AIM: To characterize genomic DNA flanking IS900 insertions and develop a rapid Multiplex PCR IS900 Locus (MPIL) typing method for *MAP* reporting the presence or absence of the element at each locus.

METHODS: Genomic DNA flanking 14 of the 18 IS900 loci was sequenced and compared with database homologues. An MPIL typing method was developed u sing a common IS900 primer and individual locus specific primers designed to produce amplification products differing by about 50 bp which could be easily resolved on a single gel. MPIL was applied to a panel of 81 *MAP* isolates and compared with RFLP profiles.

RESULTS: Genes flanking IS900 loci included homologues of transcription regulators, a sigma factor, a nitrate reductase, a polyketide synthase and an O6-methylguanine-methyl transferase. MPIL rapidly and consistently identified 10 individual types of *MAP* from the panel of 81 isolates, and distinguished between bovine and ovine strains. Nine MPIL types corresponded directly to single RFLP types previously identified.

CONCLUSION: IS900 insertions in *MAP* may affect the expression of genes critically associated with the pathogenic phenotype. MPIL typing can identify bovine and ovine strains independent of the need for culture and may contribute to studies of the molecular epidemiology of these difficult organisms.

Key words: *Mycobacterium avium*; Paratuberculosis; Polymerase chain reaction; Geneome; Gene expression

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