Isolation and Sequencing of Insertion Element IS*1311* from *Mycobacterium avium* subsp. *paratuberculosis*

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Abstract

We describe a screening strategy for a *M. a. paratuberculosis* genomic library resulting in the isolation of a clone bearing IS*1311*. Sequencing of this clone has revealed an insertion element with very high homology to the *M. avium* sequence. Sequence differences in this element between sheep and cattle strains were used to examine *M. a. paratuberculosis* from deer. Both sheep and cattle strains could be isolated from deer.

Introduction

Mycobacterium avium subsp. *paratuberculosis* has been known to be the causative agent of Johne's disease for well over a hundred years, yet there is very little information on the epidemiology of the disease. This lack of information is due to a number of factors such as the difficulty in culturing *M. a. paratuberculosis,* the very slow progression of the disease, and the lack of a robust and highly discriminating typing system for *M. a. paratuberculosis* isolates.

The discovery of the *M. a. paratuberculosis*-specific insertion element IS*900* (1) has allowed for efficient species identification. Extensive investigation of the IS*900* sequence, insertion site, and restriction endonuclease digestion has however yielded little information useful for identifying individual isolates.(2)

Current strain differentiation systems divide the species into two broad groups; namely the "Ovine" and "Bovine" types, within which there are a number of subtypes.(2) This level of differentiation is of little value when a detailed investigation of the spread and transmission of Johne's disease is desired, where a unique identification system for each isolate would be ideal.

It was with the aim of identifying a genetic element in *M. a. paratuberculosis* possessing sufficient variability to be used as an epidemiological marker that a genomic library was constructed and screened. One element isolated from this library (IS*1311*) was then used to investigate the strain type of isolates from South Island sheep and deer.

Materials and Methods

M. avium ss. *paratuberculosis* (Neoparasec vaccine strain) was digested with EcoR1 and cloned into λ ZAP. Plaque equivalents to four genomes were screened in duplicate with *M. a. paratuberculosis* and *M. avium* labeled genomic DNA as a probe. 117 clones were isolated that hybridised strongly with only one species of genomic DNA. Using the IS*1311* polymorphisms from (3) we typed a number of *M. a. paratuberculosis* isolates from sheep, cattle and deer. Seven clones were sequenced and the sequence elements in Table 1 identified.

Clone #	Sequence homology
16	50bp
25	IS1311
48	M.tb SCY 21D4
53	185 bp insert
76	IS900
93	IS900
105	IS900

Table 1. Sequence homology of 7 clones.

Results

The identification by DNA sequence homology of the 7 sequenced clones is shown in Table 1. The sequence of IS*1311* found in clone 25 from the Neoparasec strain of *M. a. paratuberculosis* is identical to the "bovine" type identified by Whittington et al (4), Table 2.

Strain/nucleotide	68	223	236	422	527	628
M. avium U16276	T	C	T	T	G	T
M. a. paratuberculosis (Whittington et al.)	C	C/T	C	C	A	C
M. a. paratuberculosis (Neoparasec Lone 25)	C	T	C	C	A	C

Table 2. The sequence of IS*1311* found in clone 25 from the Neoparasec strain of *M. a. paratuberculosis* is identical to the "bovine" type identified by Whittington et al.

M. a. paratuberculosis of both ovine and bovine strains could be isolated from deer, Figure 1 and Table 3.

Strain*	Source	IS1311/1 HinfI type
Neoparasec	Vaccine, Rhone Merrieux	Bovine
53950	Massey University, NZ	Bovine
GS Ov1	Sheep, South Otago, NZ	Ovine
GS Cv231	Deer, South Otago	Ovine
NC CvH1	Deer, South Otago	Ovine
Tu 2398	Deer, Canterbury, NZ	Bovine
92 1265	Sheep, Southland, NZ	Ovine

*All strains IS900-positive by PCR (P90/P91)

Table 3.

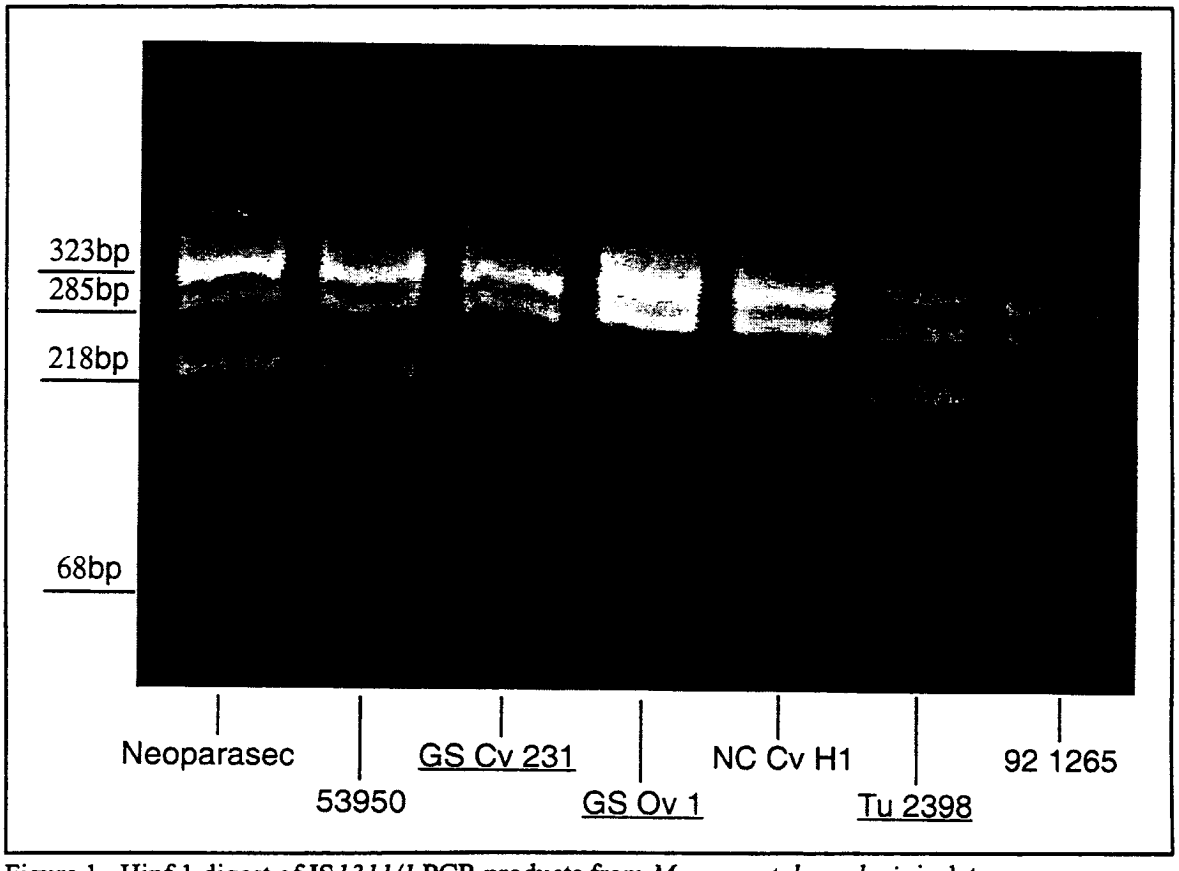

Figure 1. Hinf 1 digest of IS*1311/1* PCR products from *M. a. paratuberculosis* isolates.

Conclusion

It appears that cervids are susceptible to infection with both ovine and bovine strains of *M. a. paratuberculosis*. This finding requires further confirmation from a larger study, but would appear to reduce the disease management options available to deer farmers. The discovery of additional sequence variation within the *M. a. paratuberculosis* species is necessary if a highly discriminatory typing system is to be developed.

References

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