DNA TYPING OF RECENT NEW ZEALAND ISOLATED OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* FROM CATTLE, SHEEP AND DEEAR BY VNTR AND SSR

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Abstract
DNA typing and sub-typing of *M. avium* subsp. *paratuberculosis* (*MAP*) isolates is an important tool for any country's paratuberculosis control scheme because of its ability to answer crucial epidemiological questions, and its usefulness in infection, vaccination and pathogenicity studies. All isolates used in this study were categorised as Type C or Type S based on a specific multiplex PCR assay. A system of sub-typing based on a short sequence repeat (SSR) and variable number tandem repeats (VNTRs) that were reported to be useful in other countries was developed using 123 archival New Zealand *MAP* isolates. These isolates came from cattle, sheep and deer and included 65 Type C isolates and 58 Type S isolates. The sub-typing system was then applied to 211 recent *MAP* isolates from dairy cattle supplied mainly by the Livestock Improvement Corporation and 154 recent *MAP* isolates supplied by Massey University that came from properties where two or more ruminant species were farmed. The sub-typing system gave better discrimination of Type C isolates than Type S isolates, indicating a narrow genetic range of Type S isolates in New Zealand. There were two major sub-types of Type C, one found in 70% of dairy cattle and a few animals in mixed farming operations, and the other found in 76% of deer and 2% of dairy cattle. Unexpectedly, the majority of the 19 isolates from cattle in mixed farming operations were Type S strains. These results show that the current host distribution of Type C and Type S strains in New Zealand is substantially different from the situation that prevailed late last century indicating that these changes have occurred recently. These results also raise issues about the pathogenicity of different types and sub-types of *MAP* for different ruminant species.

Introduction
The genotyping of different strains of *MAP* provides a basis for answering important epidemiological questions about sources of paratuberculosis infection and spread of disease and enables potential variation in pathogenicity of different strains to be more easily investigated. Genotyping of New Zealand MAP isolates in the 1980s by restriction fragment length polymorphism analysis based on the insertion sequence IS*900* (1) showed that cattle and most deer were infected with Type C (later called Type II) strains while Type S (later called Type I) strains primarily infected sheep. The development of newer methods for genotyping MAP strains based on VNTR and SSR sequences now provides a faster and potentially more discriminating system that we have applied to recent New Zealand *MAP* isolates.

Methods and Results
All the MAP strains studied were determined to be Type S or Type C strains by using a specific PCR assay (2). The Type of strains found in different hosts at two time periods is given in Table 1. The most notable change over time was the recent frequent infection of cattle with Type S strains.

Table 1. Type of *MAP* strains found in different hosts over two time periods

Host	Type	1985 – 1993	2008 - 2010
Cattle	C	33	203
	S	0	27
Sheep	C	3	9
	S	16	61
Deer	C	22	62
	S	4	3

Eight VNTRs and two SSRs, reported to be particularly useful for subtyping, were applied to archived New Zealand isolates of *MAP* from cattle, deer and sheep. VNTR and SSR sequences were amplified by PCR and detected by agarose gel electrophoresis and DNA sequencing respectively. Three of the VNTRs (10, 32 and 47) that gave little or no variation and one of the SSRs (SSR1) that was too

hypervariable to give consistent results for some samples were not further used. The remaining five VNTRs (292, 25, X3, 7 and 3) and SSR8 were used to subtype 365 recent faecal isolates of MAP. These were available as the result of separate studies by the Livestock Improvement Corporation and Massey University and came either from dairy farms or from farms on which two or three different animal species (beef cattle, deer, or sheep) had been grazed. Compared to studies elsewhere, a relatively large number of the samples contained multiple subtypes (Table 2). While this was unsurprising for the mixed-farm samples that came from pools of 10-20 animals, it was not expected for the samples from dairy cattle which came from single animals.

Table 2. *MAP* samples containing more than one subtype

	Total isolates	**Samples with multiple subtypes**	**Samples with a VNTR giving 2 PCR products**	**Samples with more than 2 PCR products**
Dairy	211	30 (14%)	14	16
Mixed farms	154	10 (6%)	8	2

The results of subtyping are given in Table 3. Two subtypes of Type C predominated in dairy cattle and one of these was not found in beef cattle, deer or sheep. A different subtype of Type C predominated in deer and was also found in small numbers of other animals. While six different subtypes of Type S were found in sheep, one subtype predominated and this was also the predominant Type S subtype in other animals. Surprisingly, beef cattle were more often infected with Type S strains than with Type C strains.

Table 3. Subtyping of recent New Zealand isolates of *MAP* by five VNTRs and SSR8

Type	**Subtype 292-25-X3-7-3-SSR8**	**Dairy**	**Beef**	**Deer**	**Sheep**
Type C	2-3-2-2-2-5	1			
	3-2-2-2-2-5	25			
	3-2-2-3-2-5	2			
	3-3-2-0.5-2-5	6		3	
	3-3-2-2-2-3	1			
	3-3-2-2-2-4	4		1	2
	3-3-2-2-2-5	147	2	8	2
	3-3-2-3-2-5	1			
	4-3-2-2-2-4	5	4	51	6
	4-3-2-2-2-5	1		1	
	5-2-2-2-2-5	1			
Type S	3-3-1-1-1-3	3	1	1	5
	3-3-1-2-1-3				1
	4-3-1-1-1-3	11	12	2	50
	4-3-1-2-1-3				5
	5-3-1-1-1-3	1			2
	7-3-1-1-1-3				1

Conclusions

- While historically, NZ cattle were infected only with Type C strains, recent typing shows that Type S strains are now frequent in NZ cattle and are more common in beef cattle than are Type C strains.
- Subtyping profiles for Type C isolates were always different from those for Type S isolates and VNTR3 by itself distinguished all Type S (1 allele) from all Type C (2 alleles) isolates.
- Some subtypes of Type C are present only in dairy cattle and one subtype of Type C predominates in these animals. In contrast, a different subtype of Type C predominates in deer
- There were fewer subtypes of Type S than of Type C and a single subtype of Type S predominates in all animals. This may reflect a very narrow genetic variation of Type S strains in New Zealand

References

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