



DNA strain typing of *Mycobacterium avium* subsp. *paratuberculosis* (MAP)

Des Collins, Marian Price-Carter, Geoff de Lisle

AgResearch NCBID Wallaceville

18 - 4 - 2011

DNA strain typing

- Use of DNA methods to distinguish different *MAP* strains

Why use strain typing ?

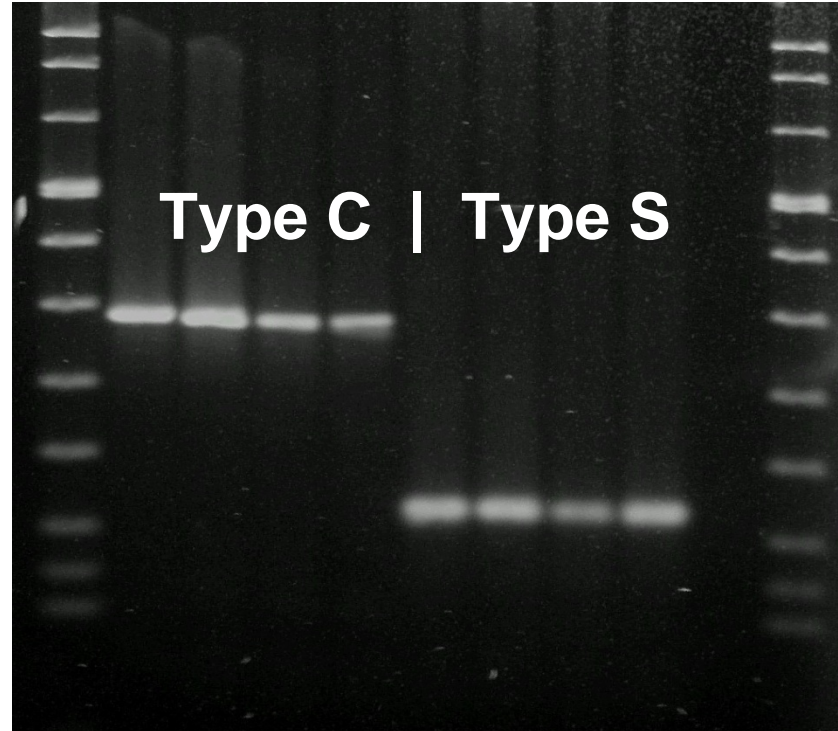
- Can provide answers to questions such as:
 - What was the source of infection for an animal or herd
 - Do different strains infect different host species
 - important in mixed farming operations
 - Does wildlife have a role in disease transmission
- Different types may have different phenotypes and this may affect:
 - Survival of strains in milk or meat
 - Disease severity in different animal species
 - interplay with host genetics
 - interplay with vaccine status
 - Disease transmission
 - association with Crohn's disease

MAP strains

- Typing with older DNA methods showed there were two different groups of *MAP* strains
- **Type C** (also called Type II)
 - NZ Pre 2010 - all infected dairy cattle and most deer
- **Type S** (also called Types I and III)
 - NZ Pre 2010 - all infected sheep and a few deer

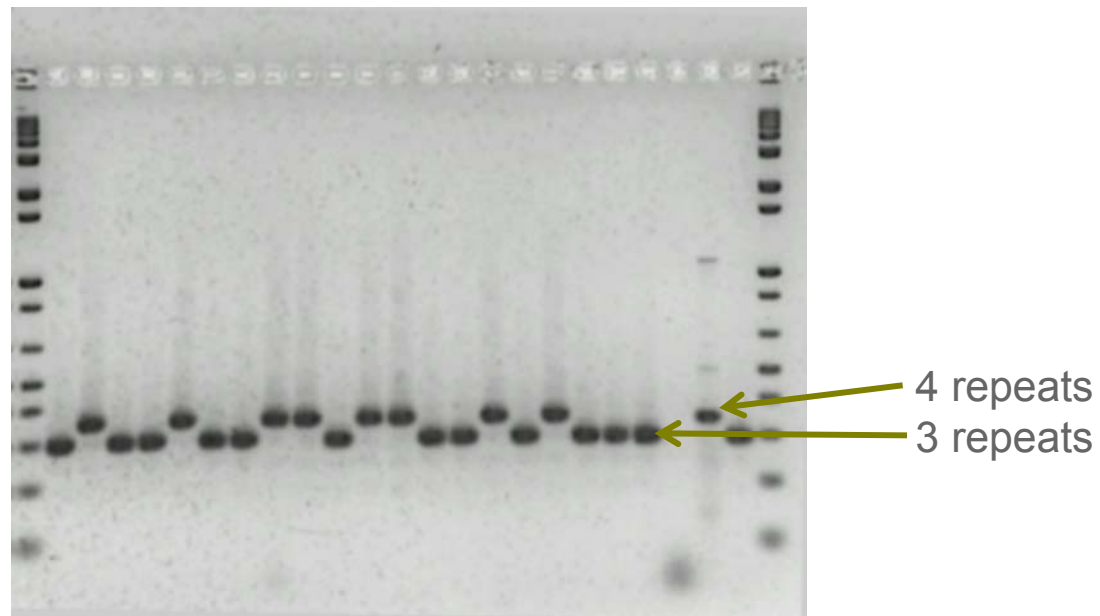
Currently using two typing methods

1. PCR assay to determine if strain is Type C or Type S



Currently using two methods

1. PCR assay to determine if strain is Type C or Type S
2. VNTR/SSR typing to give finer discrimination
(VNTR = variable number tandem repeat; SSR = short sequence repeat)



SSR typing

- SSRs are a subset of VNTRs where the repetitive sequence is only 1-3 bp long
- Requires PCR and DNA sequencing for analysis

Typing results 2008 - 2010

A. Type C/Type S determination

B. Panel of assays (5 VNTR + SSR)

1. 65 archival Type C isolates
 - Cattle and deer isolates
 - 14 VNTR/SSR types
 - 23% of isolates had the most common VNTR/SSR type.
2. 58 archival Type S isolates
 - Sheep isolates
 - 5 VNTR/SSR types
 - most isolates (90%) had the same VNTR/SSR type
3. A second SSR sequence appeared too unstable to be included as part of the typing panel

Typing in current year (July 2010 →)

1. 200 faecal isolates of MAP from dairy cattle (LIC)
2. 150 faecal isolates of MAP from mixed farming operations (Massey)
3. Stability of VNTR/SSR types during sub-culturing

Typing in current year (July 2010 →)

1. 200 faecal isolates of MAP from dairy cattle (LIC)
2. 150 faecal isolates of MAP from mixed farming operations (Massey)
3. Stability of VNTR/SSR types during sub-culturing

Project	Science report (18/3/11)	Today (18/4/11)	End of year (30/6/11)
1.	In progress	Completed	Completed
2.	Completed	Completed	Completed
3.	In progress	In progress	Completed

Typing in current year (July 2010 →)

1. 200 faecal isolates of MAP from dairy cattle (LIC)
2. 150 faecal isolates of MAP from mixed farming operations (Massey)
3. Stability of VNTR/SSR types during sub-culturing

Project	Science report (18/3/11)	Today (18/4/11)	End of year (30/6/11)
1.	In progress	Completed	Completed
2.	Completed	Completed	Completed
3.	In progress	In progress	Completed

Stability - Another SSR which gave inconsistent results is no longer being used and was not used to obtain the results given for Projects 1 and 2

Type C/Type S results for current year

Project	Type (C or S)	Dairy cattle	Beef cattle	Deer	Sheep
LIC N = 200	Type C	186			
	Type S	14			
Massey N = 154	Type C N = 81		11	62	8
	Type S N = 73		13	3	57

Type C/Type S results for current year

Project	Type (C or S)	Dairy cattle	Beef cattle	Deer	Sheep
LIC N = 200	Type C	186			
	Type S	14			
Massey N = 154	Type C N = 81		11	62	8
	Type S N = 73		13	3	57

Type C/Type S results for current year

Project	Type (C or S)	Dairy cattle	Beef cattle	Deer	Sheep
LIC N = 200	Type C	186			
	Type S	14			
Massey N = 154	Type C N = 81		11	62	8
	Type S N = 73		13	3	57

Type C/Type S results for current year

Project	Type (C or S)	Dairy cattle	Beef cattle	Deer	Sheep
LIC N = 200	Type C	186			
	Type S	14			
Massey N = 154	Type C N = 81		11	62	8
	Type S N = 73		13	3	57

Type C/Type S results for current year

Project	Type (C or S)	Dairy cattle	Beef cattle	Deer	Sheep
LIC N = 200	Type C	186			
	Type S	14			
Massey N = 154	Type C N = 81		11	62	8
	Type S N = 73		13	3	57

Mixed VNTR types in single samples

	Total	Samples with multiple VNTR types	Samples with a VNTR giving 2 PCR products	Samples with a VNTR giving 3 PCR products
LIC	200	28 (14%)	8	20
Massey	154	12 (8%)	10	2

VNTR/SSR typing - Dairy cattle (LIC)

	Total	VNTR					SSR8
		292	25	X3	7	3	
Type S N = 13	3	3	3	1	1	1	3
	10	4	3	1	1	1	3
Type C N = 159	20	3	2	2	2	2	5
	2	3	2	2	3	2	5
	1	3	3	2	0.5	2	5
	2	3	3	2	2	2	4
	131	3	3	2	2	2	5
	1	3	3	2	3	2	5
	2	4	3	2	2	2	4

VNTR/SSR typing - Dairy cattle (LIC)

	Total	VNTR					SSR8
		292	25	X3	7	3	
Type S N = 13	3	3	3	1	1	1	3
	10	4	3	1	1	1	3
Type C N = 159	20	3	2	2	2	2	5
	2	3	2	2	3	2	5
	1	3	3	2	0.5	2	5
	2	3	3	2	2	2	4
	131	3	3	2	2	2	5
	1	3	3	2	3	2	5
	2	4	3	2	2	2	4

LIC isolates 1- 9

Accession	Farm	292	25	X3	7	3	SSR8	Type C/S
W09/0539-1	A	3	3	2	2	2	5	Type C
W09/0539-2	A	3	3	2	2	2	5	Type C
W09/0539-3	A	3	3	2	2	2	5	Type C
W09/0539-4	A	3	3	2	2	2	5	Type C
W09/0539-6	A	3	3	2	2	2	5	Type C
W09/0539-7	A	3	3	2	2	2	5	Type C
W09/0539-8	A	3	3	2	2	2	5	Type C
W09/0627-2	B	3	3	2	2	2	5	Type C
W09/0627-1	B	3	234	2	2	2	5	Type C

LIC isolates 1- 9

Accession	Farm	292	25	X3	7	3	SSR8	Type C/S
W09/0539-1	A	3	3	2	2	2	5	Type C
W09/0539-2	A	3	3	2	2	2	5	Type C
W09/0539-3	A	3	3	2	2	2	5	Type C
W09/0539-4	A	3	3	2	2	2	5	Type C
W09/0539-6	A	3	3	2	2	2	5	Type C
W09/0539-7	A	3	3	2	2	2	5	Type C
W09/0539-8	A	3	3	2	2	2	5	Type C
W09/0627-2	B	3	3	2	2	2	5	Type C
W09/0627-1	B	3	234	2	2	2	5	Type C

LIC isolates 10 -18

Accession	Farm	292	25	X3	7	3	SSR8	Type C/S
W09/0626-2	C	3	3	2	2	2	5	Type C
W09/0626-1	C	4	3	1	1	1	3	Type S
W09/0600-11	D	3	3	2	2	2	5	Type C
W09/0600-5	D	3	3	2	2	2	5	Type C
W09/0600-6	D	4	3	1	1	1	3	Type S
W10/0387-1	D	4	3	1	1	1	3	Type S
W10/0412-1	E	3	2	2	2	2	5	Type C
W09/0654-1	F	3	3	2	2	2	5	Type C
W09/0654-3	F	3	3	2	2	2	5	Type C

LIC isolates 10 -18

Accession	Farm	292	25	X3	7	3	SSR8	Type C/S
W09/0626-2	C	3	3	2	2	2	5	Type C
W09/0626-1	C	4	3	1	1	1	3	Type S
W09/0600-11	D	3	3	2	2	2	5	Type C
W09/0600-5	D	3	3	2	2	2	5	Type C
W09/0600-6	D	4	3	1	1	1	3	Type S
W10/0387-1	D	4	3	1	1	1	3	Type S
W10/0412-1	E	3	2	2	2	2	5	Type C
W09/0654-1	F	3	3	2	2	2	5	Type C
W09/0654-3	F	3	3	2	2	2	5	Type C

VNTR/SSR typing - Mixed farms (Massey)

	Number of animal isolates				VNTR					SSR8
	Total	Cattle	Deer	Sheep	292	25	X3	7	3	
Type S N = 68	3	1	1	1	3	3	1	1	1	3
	1	—	—	1	3	3	1	2	1	3
	56	12	2	42	4	3	1	1	1	3
	5	—	—	5	4	3	1	2	1	3
	2	—	—	2	5	3	1	1	1	3
	1	—	—	1	7	3	1	1	1	3
Type C N = 74	3	—	3	—	3	3	2	0.5	2	5
	1	1	—	—	3	3	2	2	2	3
	14	3	9	2	3	3	2	2	2	5
	56	5	48	3	4	3	2	2	2	4

VNTR/SSR typing - Mixed farms (Massey)

	Number of animal isolates				VNTR					SSR8
	Total	Cattle	Deer	Sheep	292	25	X3	7	3	
Type S N = 68	3	1	1	1	3	3	1	1	1	3
	1	—	—	1	3	3	1	2	1	3
	56	12	2	42	4	3	1	1	1	3
	5	—	—	5	4	3	1	2	1	3
	2	—	—	2	5	3	1	1	1	3
	1	—	—	1	7	3	1	1	1	3
Type C N = 74	3	—	3	—	3	3	2	0.5	2	5
	1	1	—	—	3	3	2	2	2	3
	14	3	9	2	3	3	2	2	2	5
	56	5	48	3	4	3	2	2	2	4

VNTR/SSR typing - Mixed farms (Massey)

	Number of animal isolates				VNTR					SSR8
	Total	Cattle	Deer	Sheep	292	25	X3	7	3	
Type S N = 68	3	1	1	1	3	3	1	1	1	3
	1	—	—	1	3	3	1	2	1	3
	56	12	2	42	4	3	1	1	1	3
	5	—	—	5	4	3	1	2	1	3
	2	—	—	2	5	3	1	1	1	3
	1	—	—	1	7	3	1	1	1	3
Type C N = 74	3	—	3	—	3	3	2	0.5	2	5
	1	1	—	—	3	3	2	2	2	3
	14	3	9	2	3	3	2	2	2	5
	56	5	48	3	4	3	2	2	2	4

VNTR/SSR typing - LIC vs Mixed farms

	Source	No. of animal isolates			VNTR					SSR8
		Cattle	Deer	Sheep	292	25	X3	7	3	
Type S	LIC	9	—	—	4	3	1	1	1	3
	Massey	12	2	42	4	3	1	1	1	3
Type C	LIC	20	—	—	3	2	2	2	2	5
	LIC	131	—	—	3	3	2	2	2	5
	Massey	3	9	2	3	3	2	2	2	5
	Massey	5	48	3	4	3	2	2	2	4

VNTR/SSR typing - LIC vs Mixed farms

	Source	No. of animal isolates			VNTR					SSR8
		Cattle	Deer	Sheep	292	25	X3	7	3	
Type S	LIC	9	—	—	4	3	1	1	1	3
	Massey	12	2	42	4	3	1	1	1	3
Type C	LIC	20	—	—	3	2	2	2	2	5
	LIC	131	—	—	3	3	2	2	2	5
	Massey	3	9	2	3	3	2	2	2	5
	Massey	5	48	3	4	3	2	2	2	4

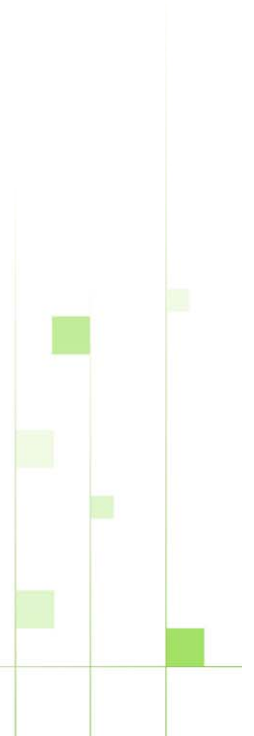
VNTR/SSR typing - LIC vs Mixed farms

	Source	No. of animal isolates			VNTR					SSR8
		Cattle	Deer	Sheep	292	25	X3	7	3	
Type S	LIC	9	—	—	4	3	1	1	1	3
	Massey	12	2	42	4	3	1	1	1	3
Type C	LIC	20	—	—	3	2	2	2	2	5
	LIC	131	—	—	3	3	2	2	2	5
	Massey	3	9	2	3	3	2	2	2	5
	Massey	5	48	3	4	3	2	2	2	4
	LIC	2	—	—	4	3	2	2	2	4

Work proposed in 2011-2012 year



1. Improve VNTR/SSR discrimination so predominant types can be further sub-divided and investigations involving typing on individual farms can give more useful results
2. Investigate role of wildlife in transmission of *MAP* strains
3. Determine if particular *MAP* types are associated with high prevalence of disease in deer herds

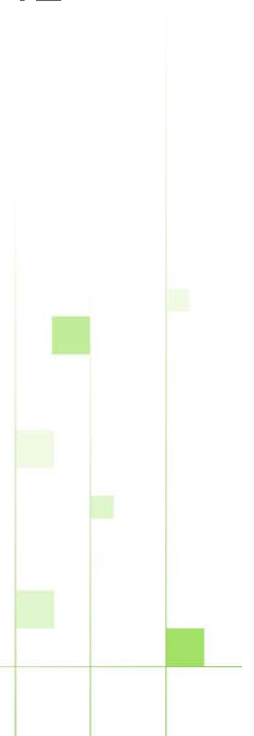


Work proposed beyond 2012



- Investigate the longitudinal changes and pathogenicity associations of *MAP* types in any new longitudinal or intervention studies carried out on dairy or mixed farms

My expectation would be that any intervention or longitudinal studies started in 2011 would involve us at the design stage so that we would get appropriate samples of *MAP* for typing in 2012



VNTR/SSR typing - LIC vs. Mixed farms

	Source	No. of animal isolates			VNTR					SSR8
		Cattle	Deer	Sheep	292	25	X3	7	3	
Type S	LIC	9	—	—	4	3	1	1	1	3
	Massey	12	2	42	4	3	1	1	1	3
Type C	LIC	20	—	—	3	2	2	2	2	5
	LIC	131	—	—	3	3	2	2	2	5
	Massey	3	9	2	3	3	2	2	2	5
	Massey	5	48	3	4	3	2	2	2	4
	LIC	2	—	—	4	3	2	2	2	4

Most common VNTR/SSR types in archival samples from deer

3	3	2	2	2	4
3	3	2	2	2	5