Molecular characterization of *Mycobacterium avium* subspecies *paratuberculosis* C-type and S-type isolated from sheep and goats by using a combination of MIRU-VNTR loci

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Abstract

Mycobacterium avium subspecies *paratuberculosis* (Map) is the etiological agent of paratuberculosis of domestic and wild ruminants. Map strains are segregated into 2 main groups or strain types referred to as sheep (S) type and cattle (C) type. Few small ruminant Map strains have been genetically characterized to date. The present study was undertaken to genetically characterize a panel of 30 small ruminant Map strains in the province of Quebec, Canada. Mycobacterial Interspersed Repetitive Units — Variable-Number Tandem Repeat analysis (MIRU-VNTR) were used as genetic markers in addition to IS1311 PCR-REA. S-type and C-type strains were found in both sheep and goats, although C-type strains were more frequently isolated from goats and S-type strains were more common in sheep. A total of 12 distinct Map genotypes were uncovered in the present collection of strains using these markers. Considering the genetic diversity reported here, molecular characterization of Map stains in small ruminants using MIRU-VNTR markers represent an interesting avenue for both epidemiological investigations regarding the sources of herd infection and association studies between Map strains and their virulence, persistence and host-specific adaptation characteristics.

Résumé

Mycobacterium avium *subspecies* paratuberculosis *(Map) est l'agent étiologique de la paratuberculose affectant les ruminants sauvages et domestiques. Les souches de Map se répartissent dans deux grands groupes ou types appelés 'sheep (S)' et 'cattle (C)'. Très peu de souches de Map provenant des petits ruminants ont été caractérisées génétiquement jusqu'à présent. Cette étude a été initiée afin de caractériser un ensemble de 30 souches de Map provenant de 5 troupeaux de moutons et 8 troupeaux de chèvres situés dans la province de Quebec, Canada, et d'évaluer leur diversité génétique. Une analyse répétée en tandem des unités répétitives alternées des mycobactéries (MIRU-VNTR) a été utilisée comme marqueurs génétiques en plus du marqueur IS1311 PCR-REA. Les souches de type S et C ont été retrouvées chez les isolats ovins et caprins, avec une prédominance des souches de type C chez les isolats provenant de chèvres tandis que les souches de type S étaient plus fréquentes chez les moutons. Un total de 12 génotypes distincts de Map ont été retrouvés parmi les isolats d'après les marqueurs utilisés. Considérant la diversité génétique observée, la caractérisation moléculaire des isolats de Map représente une avenue intéressante pour investiguer les sources potentielles d'infection des troupeaux et pour étudier les associations entre les caractéristiques génétiques et pathogéniques des isolats.*

(Traduit par les auteurs)

Introduction

Mycobacterium avium subspecies *paratuberculosis* (Map) is responsible for paratuberculosis, a chronic granulomatous enteritis of domestic and wild ruminants. The disease is prevalent in domestic animals worldwide and is associated with substantial productivity losses in cattle and small ruminants (1,2). Diagnosis and control of paratuberculosis is challenging due to the long incubation period and to the environmental resistance of Map. In domestic ruminants, paratuberculosis is generally characterized by a subclinical phase of several months or years, followed by a terminal stage eventually leading to culling or death (3). Growing evidence suggests that infection severity, the ability to survive in macrophages and the response to the host's immune system are all influenced by the type of Map strain (4–7).

Historically, Map strain types were named after the species where they were first isolated, which were the sheep (S) type and cattle (C) type (8). The C-type Map strains are the predominant strain type isolated from dairy cattle whereas S-type strains have been mainly isolated from small ruminants (9). Over the last few decades, new typing techniques were developed, mostly based on investigation of tandem-repeats (TR), which are polymorphic minisatellite sequences dispersed throughout the genome of Mycobacteria (10). Included in the TR family, the mycobacterial interspersed repetitive units

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(MIRU) and variable number tandem repeats (VNTR) methods have gained popularity due to their ease of use and improved discriminatory powers over traditional methods (11−15). Field application of these PCR-based typing methods has started to unveil interspecies transmission events of C-type and S-type strain (16,17). However, most of these studies have focused on the characterization of C-type strains due to the difficulties inherent with the isolation of S-type strains in culture.

The present study was undertaken to characterize a panel of small ruminant Map strains from different herds in the province of Quebec, Canada, and to describe their relatedness to bovine strains previously characterized in the province of Quebec. Since Map has limited genetic diversity, we used a total of 13 MIRU-VNTR markers to characterize the genome of Map strains isolated from small ruminants.

Materials and methods

Source of Map strains

Sheep — A collection of 20 strains isolated from 20 individual sheep originating from a project conducted from 2010 to 2012 involving meat sheep herds located across the province of Quebec was used. Map isolates were recovered from intestinal tissues at the ileocecal junction (ICJ) and from mesenteric lymph nodes (MLN) of sheep over 2 y of age, suspected of having paratuberculosis. Tissue sections from ICJ and MNL (0.5 cm^3) were finely chopped and suspended in 10 mL of sterile distilled water (SDW). Tissue samples were then placed in a plastic bag and homogenized using a stomacher at maximum speed for 5 min. The homogenate was mixed with 10 mL of 0.9% hexadecyle pyridinium chloride (HPC) for decontamination and allowed to stand at room temperature for 16 h. The suspension was centrifuged at 3800 × *g* for 30 min at 4°C. The pellet was resuspended in 1 mL of SDW and centrifuged at 6500 × *g* for 5 min. The final pellet was resuspended in 1 mL SDW. Aliquots of 100 μL were inoculated on 5 replicate slants of modified agar medium (Modified MiddleBrook 7H10 agar medium; BD, Mississauga, Ontario) supplemented with mycobactin and 5 replicate slants of Lowenstein-Jensen medium (BD, Mississauga, Ontario) supplemented with mycobactin (18). Slants were incubated at 37°C and observed every 2 wk for the appearance of colonies for a maximum of 36 wk. Molecular confirmation of Map strains was done using PCR (IS*900* PCR) as indicated (19).

Goat — Goat Map isolates (n = 10) originating from 10 individual goats were obtained from a collection originating from a distinct project investigating the main causes of death in goats, in Quebec in 2010 (20). A section of the ileum was collected for culture from goats presenting with gross lesions suggestive of paratuberculosis at necropsy. Granulomatous ileitis and mesenteric lymphadenitis with intra-histiocytic acid-fast bacilli, consistent with paratuberculosis, was later confirmed in all selected goats. Primary isolation of Map was done as follows: on day 1, 1 mm of ileum tissue was cut into small pieces and 400 mg were placed in a 2 mL tube containing 1 mL of saline and 1 mm glass beads. The tubes were placed for 5 min in a tissue homogenizer (Tissue lyser; Qiagen, Toronto, Ontario), and then centrifuged for 2 min at 8500 × g. The supernatant was harvested and mixed 50:50 with Brain heart infusion (BHI) broth containing 0.9% hexadecylpyridinium chloride monohydrate (HPC). The suspensions were incubated at 35°C for 24 h. On day 2, the samples were centrifuged for 30 min at 900 × *g*. The pellet was resuspended in 1.0 mL of sterile saline. For each sample, 6 tubes of mycobacteria growth indicator tube (MGIT Para TB Medium; BD) containing a different antibiotic cocktail were seeded with 100 μL of suspension. To increase the likelihood of small ruminant Map strain isolation and following discussions with the manufacturer, the tubes were incubated for 3 wk at 35°C and then transferred to the MGIT 960 apparatus for 2 consecutive cycles of 7 wk of incubation. Presumptive positive vials were confirmed by acid fast stain and by PCR (*Taq*Man Map Johne's Reagents; Life Technologies, Mississauga, Ontario). For isolating colonies, subcultures were made as follows: a total of 500 μL of growth medium from positive MGIT vials was diluted 1:5 in sterile phosphate-buffered (saline) solution (PBS). Aliquots of 100 μL of each dilution were then inoculated on 5 replicate slants of modified medium (MiddleBrook 7H10) with mycobactin (18) and egg yolk medium (Herrold's Egg Yolk medium; BD with mycobactin). Slants were incubated at 37°C and observed every 2 wk for the appearance of colonies for a maximum of 36 wk.

Group typing of Map strains using IS1311 PCR-REA

Group typing was done using IS*1311* polymerase chain reaction restriction enzyme analysis (PCR-REA) directly from Map colonies. IS*1311* PCR reactions were done as described previously with slight modifications (21,22). Briefly, single colonies were harvested with a sterile toothpick and diluted in 10 μL of PCR grade water before being heated at 95°C for 10 min. The mixture was transferred to 40 μL of a PCR reaction mix consisting of 5 μL of 10× PCR buffer, 200 μM dNTPs, 2.5 mM of $MgCl_2$, 0.6 μM of primers M-56 and M-119, and 2 U of *Taq* polymerase (Invitrogen, Mississauga, Ontario). Amplification was done under the following conditions: one cycle of 3 min at 94°C and 35 cycles of 30 s at 94°C, 15 s at 62°C, and 1 min at 72°C. Amplification reactions were analyzed on a capillary electrophoresis instrument (Qiaxcel; Qiagen). The expected amplicon size was 608 nucleotides. Digestion of the amplicons using restriction enzymes (REA reactions) were carried out in a volume of 30 μL, containing 20 μL of IS*1311* amplicons, 3 μL of reaction 10× buffer, and 10 U of each endonucleases *Hinf*I and *Mse*I (New England Biolabs, Whitby, Ontario). Reactions were incubated at 37°C for 2.5 h. Restriction reactions were analyzed using a capillary electrophoresis instrument (Qiaxcel; Qiagen). Band patterns were interpreted as previously described (21).

Genomic DNA extraction

Following months of incubation, once visible colonies were apparent on a slant, all colonies were harvested in mycobacterial storage medium (20% glycerol solution containing 3.0% w/v tryptone soya broth) by up and down pipetting and stored at −70°C. A total of 200 μL of this Map solution was used to isolate genomic DNA using a DNA extraction kit (QIAamp DNA mini kit; Qiagen) following the recommended protocol with the following slight modifications: an initial enzymatic lysis step was done using 200 μL of 20 mg/mL of lysozyme, then incubated for 3 h at 37°C before proceeding with the

Table I. Sheep Map isolate typing using MIRU-VNTR analysis.

Isolate	Farm	Group[a]	INMV profile[b]	INMV Type[c]	MV Profile[d]	MV Type[e]	Genotype[f]
JY 1	1	S	41331218	INMV 129	31112	MV 7	G 8
JY 2	2	S	41331218	INMV 129	31112	MV 7	G 8
JY 3	2	S	71331218	INMV 119	31112	MV 7	G 11
JY 4	2	C	32332228	INMV 2	31222	MV 1	G 2
JY 84	2	S	41332128	INMV 56	32222	MV 8	G 12
JY 87	2	S	41332128	INMV 56	32222	MV 8	G 12
JY 88	2	S	41332128	INMV 56	32222	MV 8	G 12
JY 91	2	S	41332128	INMV 56	32222	MV 8	G 12
JY 97	2	S	41332128	INMV 56	32222	MV 8	G 12
JY 7	3	S	41331218	INMV 129	31222	MV 1	G 9
JY 26	3	C	32332228	INMV 2	31222	MV 1	G 2
JY 37	3	C	32332228	INMV 2	31222	MV 1	G 2
JY 9	4	S	41331218	INMV 129	31212	MV 3	G 10
JY 77	4	S	40331118	INMV 61	31112	MV 7	G 5
JY 78	4	S	40331118	INMV 61	31112	MV 7	G 5
JY 79	4	S	40331118	INMV 61	31112	MV 7	G 5
JY 80	4	S	40331118	INMV 61	31112	MV 7	G 5
JY 81	4	S	40331118	INMV 61	31112	MV 7	G 5
JY 20	5	S	41331118	INMV 72	31212	MV 3	G 7
JY 46	5	C	32332218	INMV 3	31272	MV 6	G 6

[a] S — Sheep type; C — Cattle type.

[b] INMV profile based on loci: 292, X3, 25, 47, 3, 7, 10, and 32.

[c] INMV type based on the INRA MAC-INMV database for MIRU-VNTR typing.

[d] MV Profile based on loci: MIRU 1, MIRU 4, VNTR 259, VNTR 1067, and VNTR 3527.

[e] MV type sequentially following the nomenclature proposed by Sohal et al (13).

[f] Genotype based on combined INMV and MV profiles.

INMV; MV; INRA — Institut National de Recherche Agronomique, France; MAC; MIRU — mycobacterial interspersed repetitive units; VNTR — variable number tandem repeats.

manufacturer's protocol. The isolated DNA was stored at −20°C until needed.

MIRU-VNTR typing

The MIRU-VNTR typing was carried out by PCR amplification of a total of 13 different loci. A total of 8 loci [VNTR 292 (also known as MIRU-2), VNTR X3 (also known as MIRU-3), VNTR 25, VNTR 47, VNTR 3, VNTR 7, VNTR 10, and VNTR 32] described by Thibault et al (12); 2 loci (MIRU-1 and MIRU-4) described by Bull et al (24); 1 loci (VNTR 259) described by Castellanos et al (11); and finally 2 loci (VNTR 1067 and VNTR 3527) described by Overduin et al (23). For each locus, the primers and PCR conditions were exactly as described by the authors (11,15,23,24). The amplicons were analyzed on a capillary electrophoresis instrument (Qiaxcel; Qiagen). The number of repeats at each locus was determined according to the sizes of the amplicons, based on a discrimination using the number of repeats as previously described (11,23,24). The Institut National de Recherche Agronomique, France (INRA) MIRU-VNTR nomenclature (i.e., INMV profile number) was used to analyze the results for the 8 MIRU-VNTR loci: 292, X3, 25, 47, 3, 7, 10, and 32 using the INRA online typing tool *Mycobaterium avium* complex MAC-INMV, (homepage on the internet: http://mac-inmv.tours.inra.fr/). For the remaining 5 loci (MIRU 1, MIRU 4, VNTR 259, VNTR 1067, and VNTR 3527) a new nomenclature (*Mycobacterium* VNTR -MV- typing) was proposed, sequentially following the 3 different MV types reported by Sohal et al (13). The number of repeats at each of the 13 marker loci was recorded. The combination of repeats for each marker formed a specific profile for every strain, which was used as a determinant to attribute Map genotypes.

Data analysis

The allelic diversity was calculated at the different loci as previously described (25), and separately for the C-type and S-type strains. A single isolate producing the same genotype per herd was included in the calculation (25). The allelic diversity represents the probability that 2 alleles randomly selected from the population are different from each other. The distribution of genotypes at the farm level was descriptively compared between sheep and goat isolates, and also with dairy cattle strains, which were previously reported in the same province and similar period of time (13).

Results

Strain typing

A total of 20 strains were obtained from 20 individual sheep belonging to 5 distinct flocks. A total of 16 strains were S-type and 4 strains were C-type according to IS1311 PCR-REA results (Table I).

Table II. Goat Map isolate typing using MIRU-VNTR analysis.

Isolate	Farm	Group[a]	INMV profile[b]	INMV type[c]	MV profile[d]	MV type[e]	Genotype[f]
C7	a	C	42332218	INMV 5	31222	MV 1	G 3
C14	b	C	32332228	INMV 2	31222	MV 1	G 2
C29	c	C	42332228	INMV 1	31222	MV 1	G 1
C51	c	S	51130218	INMV 130	21131	MV 4	G 4
C60	c	S	51130218	INMV 130	21131	MV 4	G 4
C30	d	C	42332228	INMV 1	31222	MV 1	G 1
C41	e	C	42332228	INMV 1	31222	MV 1	G 1
C42	f	C	32332228	INMV 2	31222	MV 1	G 2
C56	g	C	42332228	INMV 1	31222	MV 1	G 1
C64	h	C	42332228	INMV 1	31222	MV 1	G 1

[a] S — Sheep type; C — Cattle type.

[b] INMV profile based on loci: 292, X3, 25, 47, 3, 7, 10, and 32.

[c] INMV type based on the INRA MAC-INMV database for MIRU-VNTR typing.

[d] MV profile based on loci: MIRU 1, MIRU 4, VNTR 259, VNTR 1067, and VNTR 3527.

[e] MV type sequentially following the nomenclature proposed by Sohal et al (13).

[f] Genotype based on combined INMV and MV profiles.

INMV; MV; INRA — Institut National de Recherche Agronomique, France; MAC; MIRU — mycobacterial interspersed repetitive units; VNTR — variable number tandem repeats.

The S-type strains grew on both MB7H10 and LJ media, whereas C-type strains only grew on MB7H10. Growth rate of S-type strains was much slower than C-type strains, taking an average of 12 wk longer before the appearance of visible colonies.

A total of 12 strains from 12 individual goats originating from 8 flocks were isolated in MGIT and confirmed by PCR. From this total, 10 samples from 8 flocks gave visible colonies on MB7H10 and HEYM media and are referred to as strains in Table II. Group typing of these Map strains using IS1311 PCR-REA revealed that the C-type represented the predominant group (8/10 strains; Table II), whereas 2 strains originating from the same flock, were S-type.

Subtyping

A total of 12 C-type strains were obtained from 8 goats and 4 sheep. The INMV typing segregated the C-type strains into 4 different INMV types: INMV 1 (n = 5), INMV 2 (n = 5), INMV 3 (n = 1), and INMV 5 (n = 1) (Tables II and III). Two distinct MV types were observed for C-type strains: MV 1 (n = 11) and MV 6 (n = 1). By combining the results of INMV and MV types, a total of 4 distinct genotypes were observed: G1 (n = 5), G2 (n = 5), G3 (n = 1), and G6 (n = 1). Only genotype G2 was shared between sheep and goats.

A total of 18 S-type strains were isolated from 16 sheep and 2 goats. A total of 6 different INMV types were observed (Tables II and III): INMV 56 (n = 5) and INMV 61 (n = 5), INMV 72 (n = 1), INMV 119 (n = 1), INMV 129 (n = 4), and INMV 130 (n = 2). Among the INMV types, INMV 56, and INMV 61 had never been reported before. Five distinct MV types were observed for S-type strains: MV 1 (n = 1), MV 3 (n = 2), MV 4 (n = 2), MV 7 (n = 8), and MV 8 (n = 5). Combined diversity analysis of INMV and MV types segregated S-type strains into 8 different genotypes: G4 (n = 2), G5 (n = 5), G7 (n = 1), G8 (n = 2), G9 (n = 1), G10 (n = 1), G11 (n = 1), and G12 (n = 5). No genotype was shared between sheep and goats.

Allelic diversity

As shown in Table III, most INMV and MV markers were monomorphic for C-type strains; only loci INMV 10, INMV 292, and MV 1067 showed heterogeneity and a total of 4 distinct genotypes were revealed. The gene diversity ranged from 0.18 to 0.54 for these 3 loci. This is in sharp contrast with S-type strains, which were polymorphic at most loci, revealing a total of 8 distinct genotypes. Only loci INMV 32 and INMV 47 were monomorphic for S-type strains. The allelic diversity ranged from 0.22 to 0.55 for the polymorphic loci.

Comparison with dairy cattle strains

The comparison of small ruminant Map strains with dairy cattle strains from the same province and similar time period revealed G2 as the shared genotype between the 3 species. The G2 genotype was also the most common among dairy cattle (Table IV). Genotype G1, which was the most commonly found genotype in goats, was also shared between goats and dairy cattle.

Discussion

Compared to Map strains originating from cattle, which are almost exclusively C-type, characterization of the genetic diversity of small ruminants Map strains is not well-documented (15). Furthermore, few studies have reported molecular characterization of S-type Map strains, most likely due to the challenge associated with their isolation and their slower growth rate than C-type strains in culture (15,26–28). In this study, a total of 30 small ruminant Map strains were isolated and genotyped, including 18 S-type strains. These strains were from a convenience sample of a limited number of farms, limiting our ability to apply the results to the entire province. However, the farms of origin were distributed across the province of Quebec, were independently owned, and typical of the small

Table III. Relative frequency (%) of selected isolates from sheep and goats according to the specific allele copy number and allelic diversity for each locus, by type (C *versus* S).

Locus	Number of isolates	Specific allele copy number										Allelic diversity
		0	1	2	3	4	5	6	7	8	≥ 9	
Type = C												
INMV												
X3	11			100								0.00
3	11			100								0.00
7	11			100								0.00
10	11		18	82								0.32
25	11				100							0.00
32	11									100		0.00
47	11				100							0.00
292	11				45	55						0.54
MV												
MIRU 1	11				100							0.00
MIRU 4	11		100									0.00
259	11			100								0.00
1067	11			91					9			0.18
3527	11			100								0.00
Type = S												
INMV												
X3	9	11	89									0.22
3	9	11	78	11								0.41
7	9		33	67								0.50
10	9		89	11								0.22
25	9		11		89							0.22
32	9									100		0.00
47	9				100							0.00
292	9					78	11		11			0.41
MV												
MIRU 1	9			11	89							0.22
MIRU 4	9		89	11								0.22
259	9		56	44								0.55
1067	9		67	22	11							0.55
3527	9		11	89								0.22

INMV; MV; MIRU — mycobacterial interspersed repetitive units.

ruminant industry, which could be considered representative of the province.

Group typing revealed that both goats and sheep can be infected with either group of strain (S-type or C-type) (4). However, the C-type was predominant in goats, whereas the S-type was most often isolated from sheep; these results are consistent with current knowledge (17,21,29,30). However, it should be noted that in our study, the protocol used for primary isolation of Map in goats was adapted for C-type strains. Considering that S-type strains have different culture requirements and often need longer incubation time (31), they may have been under detected in goat herds in this study. It would, therefore, be advisable in future studies to include isolation media that supports the growth of both C-type and S-type strains.

Two of the 3 sheep farms with C-type strains had a G2 genotype profile (INMV 2-MV1), which was also found to be the most common profile among dairy cattle strains in this province (13). Genotype G2 was also found in 2 of the 8 goat farms in the present study. Likewise, the most common genotype found among goat strains was INMV 1-MV1 (G1), which was previously determined to be common among dairy cattle in the province (13). The 2 VNTR types, INMV 1 and INMV 2, were also found to be the most common in cattle from other Canadian provinces (32) and in dairy goats from Ontario (33). Taken together, these findings could suggest the presence of a common source of infection or inter-species transmission. Further studies are warranted to characterize epidemiological links between cattle, goats, and sheep farms in this country. The presence

Table IV. Distribution of Map genotypes in 65 dairy cattle, 5 sheep and 8 goat farms.

INMV		MV			Number of positive farm[a]		
Profile	Type	Profile	Type	Genotype	Cattle[b]	Sheep	Goat
Group = C							
42332228	INMV 1	31222	MV 1	G1	5	—	5
32332228	INMV 2	31222	MV 1	G2	42	2	2
42332218	INMV 5	31222	MV 1	G3	—	—	1
32332218	INMV 3	31272	MV 6	G6	—	1	—
32332218	INMV 3	31222	MV 1	G13	17	—	—
32332228	INMV 2	31212	MV 3	G14	2	—	—
32332328	INMV 8	31212	MV 3	G15	1	—	—
22332228	INMV 13	31222	MV 1	G16	3	—	—
Group = S							
51130218	INMV 130	21131	MV 4	G4	—	—	1
40331118	INMV 61	31112	MV 7	G5	—	1	—
41331118	INMV 72	31212	MV 3	G7	—	1	—
41331218	INMV 129	31112	MV 7	G8	—	2	—
41331218	INMV 129	31222	MV 1	G9	—	1	—
41331218	INMV 129	31212	MV 3	G10	—	1	—
71331218	INMV 119	31112	MV 7	G11	—	1	—
41332128	INMV 56	32222	MV 8	G12	—	1	—
Group = B							
22532228	INMV 68	31232	MV 2	G17	2	—	—

[a] A farm was considered as positive for a genotype when the genotype was found in at least 1 animal (and/or environmental sample for dairy cattle) from the farm. A total of 191, 20, and 10 isolates were genotyped from dairy cattle, sheep, and goat samples, respectively.

[b] From Sohal et al (13).

INMV; MV — microbacterial interspersed repetitive units.

of multiple Map strain types was observed in all 5 farms harboring more than 1 isolate, suggests multiple sources of infection. This finding underscores the importance of including multiple isolates from each farm when conducting molecular epidemiology studies of Map in small ruminants.

Markers VNTR 32 and 47 were found to be monomorphic in this study in both C-type and S-type strains isolated from both species. This is consistent with a number of studies, including a recent one from our group, where nearly 200 cattle Map strains were genotyped (13,15,28). However, others have found variations at those 2 loci in populations of different animal species (12,25). Of note, the MV loci were highly monomorphic for C-type strains of sheep and goats of this study with a single locus exhibiting genetic variability. This is similar to our previous findings regarding C-type strains from cattle where only locus MV 1067 showed genetic variability (13). However, this is in sharp contrast with the genetic variability of S-type strains from this study where all 5 MV loci were found to be polymorphic. With regards to the discriminatory hierarchy, locus INMV 292 showed the highest diversity for C-type strains whereas locus MV 1067 displayed the highest variability for S-type strains. Together, these findings justify a careful evaluation of different markers and geographical specificities regarding Map strains and genetic variations. Otherwise, there is a potential risk to lose important genetic and epidemiological information. In a recent study, 24 S-type strains from various geographical areas were analyzed and only 3 INMV loci (292, X3, and 25) were found to be both polymorphic and sufficient to describe the entire variability of their strains (15). Conversely, our findings revealed that multiple markers were polymorphic and informative. Overall, MIRU-VNTR genotyping proved to be a powerful technique for discriminating Map strains in this study, particularly for S-type. According to our findings, it could be used for future molecular epidemiological studies. The discriminatory power can be further improved, by using SSR markers in conjunction to MIRU-VNTR as shown previously (13).

Overall, the results presented in this study provide additional information about the diversity of Map strains in small ruminants in Quebec. By using informative molecular markers applied to isolates infecting different host species, this study reports on the genetic diversity of a number of Map strains isolated from small ruminants. The methods presented here, in which Map strains can be relatively easily segregated based on their genotype, could serve as a starting point for investigating transmission patterns, virulence, persistence, and clinical significance of distinct Map strains in Quebec. With the recent advances and availability of whole genome sequencing technologies and the underlining promise of increased discriminatory power, future molecular epidemiology studies, as well as host-pathogen investigations, will undoubtedly unveil unprecedented findings about this important pathogen.

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