Molecular epidemiological analysis of *Mycoplasma bovis* isolates from the Pennsylvania Animal Diagnostic Laboratory showing genetic diversity

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ABSTRACT

We have examined the genetic variability of *Mycoplasma bovis* strains submitted to the Pennsylvania Animal Diagnostics Laboratory, University Park (PA-ADL), between December 2007 and December 2008. Of 4,868 total samples submitted for *Mycoplasma* testing, 302 were determined to be culture positive. *Mycoplasma bovis* (63.6%), *Mycoplasma californicum* (7.3%), *Mycoplasma bovirhinis* (2.7%), *Mycoplasma bovigenitalium* (0.7%), *Mycoplasma alkalescens* (4.9%), *Mycoplasma putrefaciens* (0.3%), and *Mycoplasma dispar* (1.3%) and unidentified *Mycoplasma* sp. (19.2%) were identified using PCR. *Mycoplasma bovis* represented the largest portion of the positive samples submitted. Each of the 192 *M. bovis* isolates was examined for variations in the *Bgl*II and *Mfe*I restriction sites of the DNA using amplified fragment length polymorphism fingerprinting and subsequently compared with the *M. bovis* type strain PG45 (ATCC 25523). Similarity between strains was calculated using the Dice similarity coefficient, which ranged from approximately 0.7 to 1.0. When clustering the isolates at greater than 95% similarity, it was determined that 11 distinct clusters were present. The results are consistent with the existence of at least 2 clonally distinct groups. No clear geographical, month of isolation, or source origination relationship was identified, indicating that a currently unclassified characteristic is responsible for the strain heterogeneity. These data indicate strong heterogeneity of *M. bovis* isolates submitted to PA-ADL. Additionally, multiple sites throughout Pennsylvania had isolates of separate clonal lineages present concomitantly, indicating the ability of multiple overlapping outbreaks to occur at a single location. *Mycoplasma bovis* represents the largest portion of *Mycoplasma* species isolated from PA-ADL samples. We propose that amplified fragment length polymorphism may serve as a valuable tool for molecu-

lar characterization of *M. bovis* strains from the United States.

Key words: cattle, epidemiology, molecular characterization, *Mycoplasma bovis*

INTRODUCTION

Mycoplasma bovis is an important pathogen in the cattle industry. It is associated with multiple disease conditions, production losses, treatment expenses, increased case fatality rates, and high prevalence rates (Nicholas et al., 2000; Nicholas and Ayling, 2003). Diseases associated with *M. bovis* include mastitis, pneumonia, arthritis, otitis, and conjunctivitis, all resulting in significant economic losses in both the dairy and meat industries (Miller et al., 1993; Seegers et al., 2003). Although *M. bovis* is sufficient to cause bovine respiratory disease, the major role it plays in pneumonia is in co-infections with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Arcanobacterium pyogenes*, leading to high morbidity and mortality. *Mycoplasma bovis* is thought to act as a predisposing factor, weakening the host immune system and leading to invasion by other pathogenic bacteria or viruses (Rosengarten and Citti, 1999). It has been estimated that one-quarter to one-third of pneumonia-related illnesses of growing cattle may be attributed at least in part to *M. bovis* infections (Nicholas et al., 2000). Cattle infected with *M. bovis* may shed the bacteria from the respiratory tract for many months, thus serving as a reservoir of infection (Nicholas and Ayling, 2003).

When combining direct and indirect costs, the total economic burden of *M. bovis*-associated mastitis and losses due to diminished carcass values is estimated to be greater than \$2 billion annually in the United States (Miller et al., 1993; Rosengarten and Citti, 1999; Nicholas et al., 2000; Smith and Hogan, 2001). This makes it the most expensive production disease of dairy herds in developed countries (Miller et al., 1993; Smith and Hogan, 2001; Seegers et al., 2003).

The impact of *M. bovis* disease upon trade and cattle movement is of particular concern and indicates the

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Table 1. *Mycoplasma* species identified from samples submitted to the Pennsylvania Animal Diagnostics Laboratory between December 1, 2007, and November 30, 2008

Species	Collection site			Total, n (%)
	Milk ¹	Lung	Other ²	
<i>Mycoplasma bovis</i>	155	33	4	192 (63.6)
<i>Mycoplasma californicum</i>	21	1	—	22 (7.3)
<i>Mycoplasma alkalescens</i>	13	1	1	15 (4.9)
<i>Mycoplasma bovirhinis</i>	—	8	—	8 (2.7)
<i>Mycoplasma dispar</i>	4	—	—	4 (1.3)
<i>Mycoplasma bovigenitalium</i>	1	1	—	2 (0.7)
<i>Mycoplasma putrefaciens</i>	1	—	—	1 (0.3)
Unknown	46	8	4	58 (19.2)
Total	241	52	9	302

¹Quarter milk and bulk tank.²Tympanic, carpus joint, nasal swab, respiratory swab, and trachea.

need to monitor the spread of *M. bovis* in cattle. Several DNA-based typing methods have been used for *M. bovis* intraspecies differentiation. They include pulse-field gel electrophoresis (PFGE), random amplified polymorphic DNA, insertion sequence profiling, restriction endonuclease analysis, and amplified fragment length polymorphism (AFLP) (Kokotovic et al., 1999; Kusi-luka et al., 2000; McAuliffe et al., 2004; Miles et al., 2005). Currently, no gold-standard molecular typing method exists for *M. bovis*, but other studies have demonstrated the suitability of AFLP as a directly comparable inter-laboratory typing method that allows for a highly discriminatory, high-throughput, reproducible, and robust analysis (Kusi-luka et al., 2000; McAuliffe et al., 2004). Analyses by AFLP from the United Kingdom and Denmark have shown distinct *M. bovis* strain clusters and highly homogeneous clusters, respectively (Kusi-luka et al., 2000; McAuliffe et al., 2004). This study was designed to assess the genetic relatedness of *M. bovis* isolates (n = 192) submitted to the Pennsylvania Animal Diagnostic Laboratory (PA-ADL) over a 1-yr period (2007–2008) using AFLP analysis.

MATERIALS AND METHODS

Isolation and Identification of *Mycoplasma bovis*

A total of 4,868 samples were submitted to PA-ADL over a 1-yr period (December 2007 to November 2008). All *Mycoplasma*-positive samples, as determined by a positive culture (n = 302), submitted to the PA-ADL were grown on a pleuropneumonia-like organism (PPLO) agar plate (UC Davis Biological Media Services, Davis, CA) at 37°C under microaerophilic conditions until growth was noted (Table 1). Under a light microscope, a small section of the agar was identified that contained fewer than 5 *Mycoplasma* colonies and no gross contamination from other bacteria. The section

was excised from the agar and placed in PPLO broth (UC Davis Biological Media Services) for 48 h at 37°C under microaerophilic conditions. The resulting broth suspension was plated again and the process repeated. The final broth suspension was stored in 10% glycerol at –80°C until further analysis.

Then, DNA was extracted from 15- to 25-mL aliquots of stationary-phase cells using the phenol-chloroform procedure described previously (Pospiech and Neumann, 1995; Sambrook and Russell, 2001). Briefly, the culture pellet was suspended in a Tris-EDTA solution (pH 7.5) with lysozyme and treated with proteinase K before addition of phenol-chloroform. The aqueous phase was washed multiple times using ethanol and resuspended in sterile water. All DNA extracts were quantified spectrophotometrically. Isolates were confirmed as *M. bovis* using specific primers for the *uvrC* gene of *M. bovis* (Thomas et al., 2004). The isolates determined not to be *M. bovis* were confirmed as *Mycoplasma dispar* (Miles et al., 2004; Marques et al., 2007), *Mycoplasma bovirhinis* (Miles et al., 2004), *Mycoplasma putrefaciens* (Nicholas et al., 2008), *Mycoplasma bovigenitalium* (Baird et al., 1999; Nicholas et al., 2008), and *Mycoplasma californicum*, *Mycoplasma canadense*, or *Mycoplasma alkalescens* (Baird et al., 1999) based upon the PCR procedures and product sizes described previously. Based upon PCR speciation, 192 isolates were shown to be *M. bovis*. The *M. bovis* standard strain PG45 (accession number 25523) obtained from the American Type Culture Collection (ATCC, Manassas, VA) was used as a comparison strain. The strains *M. californicum* (ATCC 33461), *M. bovigenitalium* (ATCC 19852), and *M. dispar* (ATCC 27140), were also used in standardizing the PCR protocols.

Samples from Pennsylvania, Vermont, and Maryland were examined for *M. bovis*. A total of 18 counties were represented in Pennsylvania (n = 186), 1 in Maryland (n = 1), and 1 in Vermont (n = 5). Multiple sites of

Table 2. Origins and characteristics of *Mycobacterium bovis* isolates by amplified fragment length polymorphism (AFLP) pattern

AFLP type at 95% clustering	Geographical origin ¹	Collection source ²	Month of collection ³
A	Addison, VT (n = 5)	2	b, c, d, e, f
	Blair, PA (n = 102)	1, 2, 4	a, b, c, d, e, f, g, h, i, j, k, l
	Bradford, PA (n = 2)	2	b, c
	Centre, PA (n = 1)	2	b
	Chester, PA (n = 2)	1	h
	Clarion, PA (n = 1)	2	b
	Franklin, PA (n = 1)	2	c
	Fulton, PA (n = 1)	2	e
	Garrett, MD (n = 1)	1	h
	Huntingdon, PA (n = 13)	1, 2	b, c, h
	Indiana, PA (n = 1)	1	b
	Lancaster, PA (n = 1)	4	g
	Montgomery, PA (n = 2)	1	a, c
	Montour, PA (n = 1)	6	f
	Perry, PA (n = 2)	1	d
	Somerset, PA (n = 2)	2	b, c
	Sullivan, PA (n = 1)	2	e
	Adams, PA (n = 1)	1	d
B	Blair, PA (n = 1)	1	k
C	Huntingdon, PA (n = 1)	1	l
D	Blair, PA (n = 30)	1, 2	b, e, f, i, j, k, l
	Bradford, PA (n = 1)	2	b
	Clarion, PA (n = 1)	2	i
	Clinton, PA (n = 1)	5	i
	Huntingdon, PA (n = 3)	1, 4	b, j, l
	Lancaster, PA (n = 1)	1	l
E	Blair, PA (n = 4)	1	c, i, j
	Huntingdon, PA (n = 1)	1	b
F	Blair, PA (n = 1)	3	f
	Crawford, PA (n = 1)	2	h
	Sullivan, PA (n = 1)	2	d
G	Fulton, PA (n = 1)	2	f
H	Somerset, PA (n = 1)	2	d
I	Crawford, PA (n = 1)	2	i
J	Blair, PA (n = 1)	1	j
K	Blair, PA (n = 1)	1	l

¹County and state.²Collection source: 1 = milk; 2 = lung; 3 = tympanic; 4 = bulk tank; 5 = carpus joint; 6 = nasal swab.³Month: a = December 2007; b = January 2008; c = February 2008; d = March 2008; e = April 2008; f = May 2008; g = June 2008; h = July 2008; i = August 2008; j = September 2008; k = October 2008; l = November 2008.

infection including udder (n = 151), lung (n = 33), tympanic cavity (n = 2), carpus joint (n = 1), nasal swab (n = 1), and bulk tank (n = 4) were represented by the isolates (Table 2).

AFLP Analysis

Digestion, ligation, and amplification of the DNA were carried out as described previously (Kokotovic et al., 1999). Briefly, the template DNA was simultaneously digested with 5 U of *Bgl*II and 5 U of *Mfe*I (New England Biolabs, Ipswich, MA) at 37°C for 2 h in digestion buffer containing 10 mM Tris-acetate, 10 mM magnesium acetate, 50 mM potassium acetate, 5 mM dithiothreitol, and 50 ng of BSA. Total reaction volumes were 20 µL each. A 5-µL aliquot of the DNA

digest was added to 15 µL of ligation mix containing 2 pmol of *Bgl*II adapter, 20 pmol of *Mfe*I adapter, 1 U of T4 DNA ligase (New England Biolabs), 2 µL of ligase buffer (New England Biolabs), and 8 µL of restriction buffer. Total ligation volume was 20 µL, and the ligation reaction was carried out for 24 h at room temperature. The modified genomic fragments were amplified with a *Bgl*-2F-0 primer labeled with a 5'-6 carboxyfluorescein (FAM) and an *Mfe*-I-0 primer as described previously (Kokotovic et al., 1999). The PCR was performed in a 25-µL total reaction volume containing 4 µL of ligation product, 0.2 µmol of each deoxynucleoside triphosphate, 2.5 mmol of MgCl₂, standard polymerase buffer (New England Biolabs), 100 ng of each primer, and 1.5 U of Taq polymerase (New England Biolabs). The cycling conditions included an initial denaturation step

of 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 54°C for 60 s, extension at 72°C for 90 s, and a final extension step of 72°C for 10 min. Amplification products were detected alongside a LIZ500 size standard at the Pennsylvania State University Genomics Core Facilities (University Park, PA) under previously described conditions (Kokotovic et al., 1999). Eight isolates were selected and run in triplicate to verify the ability to accurately reproduce results. The *M. bovis* standard strain was run a total of 7 times, with at least 1 replicate per plate submitted for fragment analysis. Additionally, ATCC strains for *M. californicum* (33461), *M. bovisgenitalium* (19852), and *M. dispar* (27140) were selected to compare against outlying *M. bovis* samples.

Data Analysis

Data collection, curve conversion, and pattern analysis were performed using GelCompar 6.0 (Applied Maths, Austin, TX). The level of similarity between fingerprints was calculated using the band-based Dice similarity coefficient (S_D). Dendrograms were created using the unweighted pair group method with arithmetic means (UPGMA). Branch resampling support was conducted using the cophenetic correlation coefficient, which determines how accurately a dendrogram preserves the pairwise distances of the unmodeled data with values ranging from 60 to 100.

RESULTS

In total, 4,868 samples were submitted to PA-ADL for *Mycoplasma* diagnosis between December 1, 2007, and November 30, 2008. Quarter milk and bulk tank milk samples represented 95.5% of samples submitted. The other 4.5% of samples were from various collection sites including tympanic, ear swab, nasal swab, nasal wash, lung, tracheal wash, carpus joint, ocular swab, pharyngeal swab, endotracheal wash, transtracheal wash, mammary tissue, and urine. Samples submitted were from bovine, canine, caprine, cervine, feline, and ovine.

A total of 302 (6.2%) of all samples submitted for *Mycoplasma* testing were positive. *Mycoplasma bovis* represented the largest percentage of species isolated from samples ($n = 192$). *Mycoplasma bovis* isolates were from bovine ($n = 191$) and cervine ($n = 1$). January and November 2008 represented the months with the highest submission of samples (>600). Other bovine *Mycoplasma* species represented included *M. californicum* ($n = 22$), *M. bovirhinis* ($n = 8$), *M. bovisgenitalium* ($n = 2$), *M. alkalescens* ($n = 15$), *M. putrefaciens* ($n = 1$), *M. dispar* ($n = 4$), and unknown ($n = 58$) (Table 1).

A total of 5 of the unknown samples were isolated from canine, feline, and caprine sources. *Mycoplasma bovis* was the species identified most frequently (64%) in the quarter and bulk tank milk samples ($n = 241$). Of the quarter milk and bulk tank milk samples *M. californicum*, *M. alkalescens*, *M. bovisgenitalium*, *M. putrefaciens*, and *M. dispar* represented 8.7, 5.4, 0.4, 0.4, and 1.7%, respectively (Table 1). *Mycoplasma bovis* (63.5%) was also the most frequently isolated species from lung samples. *Mycoplasma bovirhinis*, *M. bovisgenitalium*, *M. californicum*, and *M. alkalescens* represented 15.3, 1.9, 1.9, and 1.9% of lung isolates, respectively (Table 1).

The similarity between individual fingerprints, calculated by the Dice similarity coefficient, ranged from 0.70 to 1.0. A total of 192 *M. bovis* isolates and the PG45 standard strain were typed with AFLP, resulting in 39 profiles at $\geq 99\%$ similarity. When comparing manually clustered AFLP fragments sized 100 to 490 bp at 95% similarity, the isolates could be separated into 11 clusters (A to K). Clusters A and D contained 136 and 37 isolates, respectively (Figure 1), and the standard strain clustered within A. The similarity between the most distantly related clusters, A and K, was 70.5%. No temporal, geographical, or source origination relationship to AFLP patterns was found.

From 250 to 400 bp, a difference of 10 (± 2 bands) bands was found between the isolates in clusters A and D, with 40 bands and 50 bands, respectively. Strong genetic heterogeneity was observed for 3 strains, each of which had similarity values $< 80\%$. Clusters H to K exhibited the greatest genetic diversity (Figure 1). Each of these clusters contained fewer bands than the other clusters between 100 and 155 bp. Only 9 strains (3 from cluster F and 1 each from clusters C, D, G, J, I, and K) were shown to be missing both bands at positions 118 and 122. Cluster A was shown to be missing a band at position 369 that was present in all samples of clusters C and D, whereas cluster B had 1 isolate with the band present and 1 without the band.

DISCUSSION

Because *M. bovis* represented the largest portion of *Mycoplasma* species identified, the focus of this study was to determine the clonal diversity of *Mycoplasma bovis* submitted to PA-ADL. The high percentage of samples submitted from milk sources (95.5%) indicates the perceived importance of *Mycoplasma* infection in the Pennsylvania dairy industry. A total of 6.2% of all samples submitted were positive for *Mycoplasma*. Of these milk samples, 64.3% were *Mycoplasma bovis*. The next most prevalent mycoplasmal mastitis agents were *M. californicum* and *M. alkalescens*, representing 8.7 and 5.4% of milk isolates, respectively. Because of the

similarity between *M. bovis* and *M. californicum*, tetracycline testing in aerobic conditions may sometimes be necessary (Nicholas and Ayling, 2003). It has been suggested that *M. dispar* may be underreported in milk samples because of its fastidious nature (Nicholas et al., 2008). All of the *M. dispar* samples isolated were from quarter milk sources. Although *M. dispar* is present in

Pennsylvania, *M. bovis*, *M. californicum*, and *M. alkalescens* were more prevalent in milk samples. Evidence suggests that *Mycoplasma* species are capable of infecting multiple organ sites (Tables 1 and 2). These results suggest that *M. bovis* is the most important *Mycoplasma* species for cattle in Pennsylvania. Each *Mycoplasma* species identified was found at multiple geographical locations, indicating that geography is unlikely to be responsible for the presence of specific species.

Although this is the first report of *M. bovis* isolated from wildlife in Pennsylvania, the identification of *M. bovis* from a cervine lung sample has previously been reported in the United States (Dyer et al., 2004). The unknown *Mycoplasma* samples are likely represented by one of the many known mycoplasmas without specific PCR primer sets available, such as *Mycoplasma verecundum* (Nicholas et al., 2008). Few reports exist regarding the prevalence of the species without specific primer sets available for testing in diagnostic laboratories (Nicholas and Ayling, 2003; Fox et al., 2005; Nicholas et al., 2008).

Analysis by AFLP was standardized and validated in the laboratory before analysis of collected *M. bovis* isolates. The AFLP patterns produced in this study were highly reproducible, as demonstrated by selecting 8 isolates to run in triplicate and the standard strain run a total of 7 times, which returned patterns of >99% similarity, which is in agreement with the expected 0 to 2% average errors from repeated test samples (Mueller and Wolfenbarger, 1999). Previous studies have demonstrated the comparability of the discriminatory power of AFLP to PFGE for *M. bovis* isolates (Kusiluka et al., 2000; McAuliffe et al., 2004). This study represents the first use of AFLP to elucidate the genetic relatedness of strains of *M. bovis* isolated in the United States.

The Dice index was used in conjunction with the unweighted pair group method with arithmetic means because densitometric curves were converted into a band pattern for analysis. The phenogram type employed by this study is frequently used to infer epidemiological relationships (Riley, 2004). Genetic relatedness was selected at the level of 95% similarity to determine cluster relationships because it offers more flexibility than a cladistic approach while clearly defining highly homogeneous populations (Figure 1). The use of this index enabled analysis to determine if geographic location, source of infection, or time of collection were responsible for the cluster patterns indicated. It was determined that geographic origin, source of infection, and time of collection were not responsible for the patterns identified. It is likely that extensive cattle transport throughout Pennsylvania and the rest of the United States, as well as the consolidation of herds and changes in management practices, influences the spread

Figure 1. Genetic relationship between *Mycoplasma bovis* strains based upon amplified fragment length polymorphism profiles (100–490 bp fragment size range) produced by amplification of *Bgl*II and *Mfe*I DNA templates with nonselective primers. The dendrogram was produced with the unweighted pair group method with arithmetic means with the Dice similarity coefficient, and strains with greater than 95% similarity were collapsed into single clusters (A to K).

of a particular *M. bovis* pattern to farms separated by considerable distances. It would be informative to compare isolates collected over long periods and distances in the United States to determine the likelihood of transmitting strains through long-distance travel.

Use of an AFLP typing method may be useful in tracing outbreaks in the United States as well as to screen isolates from imported animals. Multiple individual sites throughout Pennsylvania had isolates from distinct clusters present at a single time point, indicating the ability of multiple overlapping outbreaks to occur at a single location. Tympanic samples collected from the same farm on the same day from 2 animals clustered in A and F (similarity ~90%), demonstrating this phenomenon.

The source of the isolates was weighted toward mastitis cases (almost 80% of isolates). *Mycoplasma bovis* isolates are more frequently associated with mastitis in the United States compared with European isolates (Kusiluka et al., 2000; McAuliffe et al., 2004). The strains submitted to PA-ADL in cluster A had a high degree of relationship to the type strain PG45 (Figure 1) and are therefore believed to be highly homogeneous with European strains previously reported to be closely related to the standard strain (Kusiluka et al., 2000; McAuliffe et al., 2004). This suggests that these *M. bovis* strains, which were isolated from mastitis cases and bulk tank milk samples, are related to the isolates from pneumonic cattle in Europe. Expanding the comparison of various laboratory and pathologic specimens to obtain additional AFLP patterns would be very informative in our understanding of the most common genetic pattern (bands and sequences) and the distribution of the isolates.

We hypothesize that clusters A, B, C, and D are from one clonal line of descent, whereas clusters E, F, G, H, I, J, and K represent another distinct lineage (Figure 1). It is also possible that clusters H, I, J, and K may represent novel clonal lineages because each is distinctly separate from the other clusters. As has been previously shown, *M. bovis* is occasionally isolated from sources other than cattle (Nicholas and Ayling, 2003). The cervine sample submitted to PA-ADL typed with group D, suggesting that host species does not play a role in the clonal descent because this isolate typed with a large group of cattle isolates. It is unlikely that these isolates represent laboratory aberrations, as we feel that we eliminated any potential source of laboratory variability. All media used for testing met quality control and assurance procedures of commercially available mycoplasma media, we used an extensive enrichment and selection process to ensure a single genetic isolate, and the strains were each classified as being *M. bovis* by PCR classification of the *uvrC* gene. When

compared with AFLP patterns for *M. bovis*, *M. dispar*, *M. bovisgenitalium*, and *M. californicum*, the 3 isolates were shown to cluster in patterns similar to that shown in Figure 1, with the ATCC strains being more closely related than the 3 isolates (clusters I, J, and K). This may be caused by the extremely small genome size (1,080 kbp) and the ability to quickly genetically alter. Certainly, it would be interesting to use this type of methodology in a comparison of pathologic conditions and disease outcomes to determine whether heterogeneity observed by AFLP analysis could account for differences in virulence, transmission, and evasion of the host's immune response.

It is possible that clusters J and K separated at approximately the same time from the clonal line of descent giving rise to clusters E to K, because of a distance of only 12 miles (19 km) between farms and 81% similarity between the 2 strains. It may be informative to compare these 2 strains acquired from quarter milk to others from other large dairy-producing states in the United States. The genomic plasticity of *M. bovis* might play a role in the heterogeneity due to recombination in repeat regions of the genome (Rocha and Blanchard, 2002). Such changes have been associated with antigenic variation and may be important for determining heterogeneity with AFLP (Kenri et al., 1999). Although previous work has not shown a connection between strain type and variable surface protein type, it is not known if US *M. bovis* strains have a relationship to variable surface protein type (McAuliffe et al., 2004). It may be useful to explore this relationship further.

In conclusion, in this study we used a novel technology, AFLP, to examine the genetic relationships of *M. bovis* isolates submitted to the PA-ADL from the northeastern United States, demonstrating the ability to separate isolates into clonally distinct groups. *Mycoplasma bovis* was the most important *Mycoplasma* species isolated from samples submitted to PA-ADL between December 1, 2007, and November 30, 2008. Isolates submitted to PA-ADL showed strong genetic heterogeneity. We have shown AFLP to be a reliable and informative tool for these types of analyses: its ease and reproducibility should permit inter-laboratory comparison of isolates. This type of analysis has the potential to evolve into the standard tool to characterize and trace various genetic isolates of pathogens in the US livestock industry.

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