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Molecular Epidemiology of *Mycobacterium bovis* in Texas and Mexico

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Seventy-nine *Mycobacterium bovis* isolates recovered from Mexican and Texas cattle were categorized into 16 and 25 distinct types on the basis of IS*6110* and direct-repeat fingerprint patterns, respectively. By using a combination of both fingerprint patterns, 30 distinct restriction fragment length polymorphism types were defined. Fifty-eight of 79 isolates (73%) were distributed among nine clusters. Clustered isolates were identified within herds, as well as in geographically disperse herds in Texas and Mexico. This observation is consistent with active transmission within herds and among herds, presumably as a result of active or historical cattle movements. The majority of bovine isolates (64 of 79) exhibited a single copy of IS*6110*. Interestingly, in contrast to previous studies, a high percentage of bovine isolates (15 of 79) exhibited multiple IS*6110* copies (two to five) distributed among 11 different restriction fragment length polymorphism types. It is speculated that transmission from noncattle sources may be responsible. Continued fingerprinting of isolates originating from nonbovine sources and herd surveys is expected to provide useful information regarding the epidemiology of tuberculosis in this region.

Mycobacterium bovis, the etiological agent of bovine tuberculosis, has been reported in a wide variety of domestic animals and wildlife (10, 18, 19, 21). Eradication schemes in developed countries over the past 50 years have resulted in significant declines in bovine tuberculosis; however, the presence of reservoir hosts, including the brush-tailed possum in New Zealand and the badger in Great Britain (1), and the importation of infected domestic animals have impeded eradication of the disease.

In the United States, the bovine tuberculosis eradication program was launched in 1917 during a period when the prevalence of the disease was estimated to be 5% in cattle and 15% in swine (6). By 1940, the incidence of bovine tuberculosis in all states had decreased to less than 0.5%. By 1991, 41 states plus the U.S. Virgin Islands were accredited as being tuberculosis free. During the past 10 years bovine tuberculosis in the state of Texas has been on the rise, and Texas now harbors more than 50% of *M. bovis*-infected U.S. cattle. The epidemiological causes of the disease are presumed to include importation of infected animals, incomplete depopulation of infected herds, movement of tuberculosis-exposed animals between herds, and transmission from unidentified wildlife reservoirs (13, 17).

Identification and differentiation of various strains by recently developed DNA marker techniques would provide a better understanding of the epidemiology of *M. bovis* infections and effective control of the disease in Texas. *M. bovis* is closely related to the other organisms of the *M. tuberculosis* complex including *M. tuberculosis*, *M. africanum*, and *M. microti* and has 85 to 100% homology at the DNA level (5, 8). Until recently, only limited strain differentiation among organisms identified as *M. bovis* was possible on the basis of the use of amino acids, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of extracted bacterial protein, and phage susceptibilities (2, 4). Among the recent DNA-based techniques, restriction enzyme analysis was the first used in epidemiological studies of *M. bovis* infections (3). Although this method yielded reproducible results, large numbers of complex DNA patterns made interpretation of the results difficult. The identification of insertion elements such as IS*6110* and IS*1081* which are specific to *M. tuberculosis* complex organisms led to the application of these elements in restriction fragment length polymorphism (RFLP) analysis in the epidemiological study of human tuberculosis. Fingerprint patterns based on IS*6110*, a 1.4-kb element related to the IS*3* family of insertion sequences, contain a high degree of polymorphism (up to 20 copies) among *M. tuberculosis* isolates. In contrast, most *M. bovis* isolates of bovine origin (97%) harbor a single IS*6110* copy (3, 10, 16, 21). Because of the limited degree of IS*6110*-associated polymorphism, other repetitive genetic elements such as polymorphic GC-rich sequences (PGRS) and direct-repeat (DR) elements are used to distinguish *M. bovis* strains harboring single copies of IS*6110* (14, 21).

In the present study, *M. bovis* isolates originating from Texas and Mexican cattle were fingerprinted by using IS*6110* and DR probes. The isolates were categorized into distinct RFLP types on the basis of a combination of individual fingerprint patterns. This method revealed that bovine tuberculosis cases in Texas and Mexico are caused by strains exhibiting different RFLP types. A significant proportion of bovine isolates harbored multiple IS*6110* copies, which is a characteristic feature of isolates originating from animals other than cattle (17). The potential for wildlife-to-bovine transmission is discussed.

MATERIALS AND METHODS

Mycobacterial isolates. The 79 bovine isolates used in the present study originated from Texas (n = 17), Mexico (n = 60), Kansas (n = 1), and Mississippi (n = 1). Seven additional cervid isolates originated from deer (n = 3) and elk (n = 4) in New York and Montana. Three human *M. tuberculosis* isolates used

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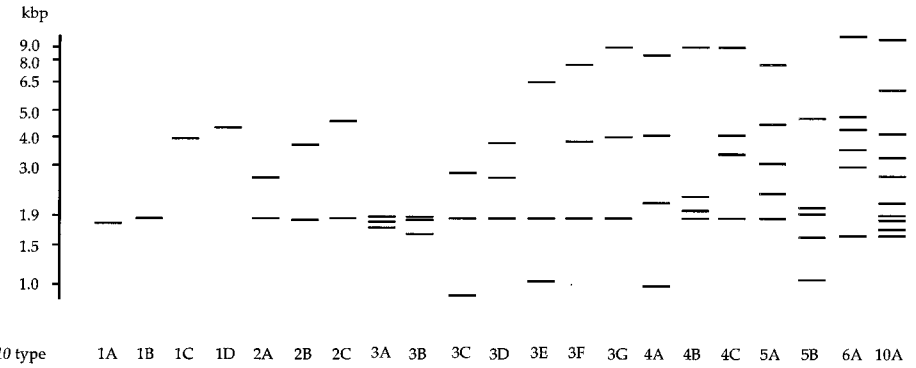

FIG. 1. IS*6110* restriction profiles. Lane maps representing 16 distinct IS*6110* fingerprint patterns exhibited by 79 bovine isolates along with two patterns exhibited by deer isolates (patterns 2A and 3C) are shown. The last three lanes (lanes 5B, 6A, and 10A) represent the fingerprint patterns of human *M. tuberculosis* isolates.

as controls were obtained from David McMurray, Department of Medical Microbiology, Texas A&M University. Seventy of the 79 *M. bovis* strains were recovered at slaughter from animals in Texas feedlots or herds. Feedlot cattle included animals which originated in either Texas, Mexico, or neighboring states and were maintained for up to 4 months. The remaining nine isolates were obtained from animals in Mexico. Fifty-one of the 70 isolates were traced back to cattle of Mexican origin, and 1 isolate each was traced back to Kansas and Mississippi. Of the 17 confirmed Texas isolates, 10 were recovered from herds restricted to Texas: the cities of Clint (n = 7), Comanche (n = 2), and Fabens (n = 1). The remaining eight isolates were recovered from animals in Texas feedlots, but the origins of these animals were not available. All *M. bovis* isolates were cultured at the National Veterinary Services Laboratories, U.S. Department of Agriculture, Ames, Iowa (n = 77), or the National Autonomous University of Mexico, Mexico City, Mexico (n = 9). *M. bovis* isolates were cultured immediately upon receipt in 20 ml of Middlebrook 7H9 broth (Difco Laboratories) supplemented with oleic acid-albumin-dextrose-catalase (OADC) enrichment medium (Difco) for 3 weeks at 37°C. The *M. bovis* isolates were further characterized on the basis of susceptibility to 5 μg of thiophene carboxylic hydrazide (Sigma Chemical Co.) per ml in Middlebrook 7H10 agarose medium supplemented with OADC (9). The cultures were harvested following heat killing at 80°C for 40 min.

DNA techniques. Mycobacterial genomic DNA was extracted as described previously by van Soolingen et al. (22). Two micrograms of genomic DNA from each isolate was digested with 10 U of either *Pvu*II (Boehringer Mannheim) for analysis with the IS*6110* probe or *Alu*I (Promega) for analysis with the DR probe (11). Genomic digests were electrophoresed through 1.2% (wt/vol) agarose in 1× TBE buffer (0.09 M Tris, 0.09 M boric acid, 0.002 M EDTA [pH 8.0]) for *Alu*I-digested DNA and 0.8% (wt/vol) agarose gels for *Pvu*II-digested DNA for 7 h in 15-cm gels at 5 V/cm. Molecular size markers included a *Pvu*II-digested supercoiled DNA ladder and *Hae*II-digested ϕX174 DNA (Gibco BRL), and these were loaded into each lane as recommended in the standard protocol for IS*6110* fingerprinting of *M. tuberculosis* isolates (20). Following electrophoresis, the DNA was partially depurinated by soaking the gel for 20 min in 0.25 N HCl. The DNA fragments were transferred onto charged nylon membranes (Hybond-N^+; Amersham) by capillary blotting in 0.4 N NaOH. Following overnight transfer, the membranes were rinsed in 2× SSPE (300 mM sodium chloride, 20 mM sodium phosphate, 0.002 mM EDTA) and air dried. The DNA fragments were cross-linked to the membranes by UV irradiation (125 mJ) in a GS Genelinker UV chamber (Bio-Rad).

A 523-bp IS*6110* fragment was synthesized by PCR with internal primers (5′-TCA GCC GCG TCC ACG CCG CCA A-3′ and 5′-CCG ACC GCT CCG ACC GAC GGT-3′) obtained from David McMurray. The PCR product was separated by electrophoresis, recovered by electroelution, and purified on NACS columns (Gibco BRL). The IS*6110* probe was labeled by the random-priming method with the Klenow enzyme and [α-^{32}P]dATP (7). A 36-bp oligonucleotide designated DR-r that was described previously (11) was labeled with terminal transferase and [α-^{32}P]dATP (15). These procedures typically yielded probes with specific activities ranging from 10^8 to 10^9 cpm/μg, and hybridizations were performed with labelled probes at 10^5 cpm/cm^2.

Southern blots containing *Pvu*II-digested genomic DNA were prehybridized in excess solution (0.5 ml/cm^2) (1.5× SSPE, 0.1% [wt/vol] SDS, 0.1% [wt/vol] sodium pyrophosphate, 0.5% [wt/vol] nonfat dried milk, 0.1 mg of herring sperm DNA per ml) for up to 8 h in a rotating chamber at 68°C and were hybridized overnight in the same buffer (50 μl/cm^2) containing labeled IS*6110* probe. The blots were washed two times each at room temperature for 15 min in 2× SSPE–0.1% (wt/vol) SDS and 1× SSPE–0.1% (wt/vol) SDS and once in 1× SSPE–0.1% (wt/vol) SDS at 50°C for 30 min. The blots containing *Alu*I-digested DNA were prehybridized and hybridized in buffer containing 6× SSPE, 0.1% (wt/vol) SDS, 0.1% (wt/vol) sodium pyrophosphate, 0.5% (wt/vol) nonfat dried milk, and 0.1 mg of herring sperm DNA per ml. These blots were washed two times each at room temperature for 15 min in 6× SSPE–0.1% (wt/vol) SDS and 3× SSPE–0.1% (wt/vol) SDS and one time in 1.5× SSPE–0.1% (wt/vol) SDS at 50°C. The washed blots were wrapped in Saran wrap and were exposed to X-ray film (Hyperfilm; Amersham) at −70°C with intensifying screens (DuPont). The blots were stripped in 0.1× SSPE–0.1% (wt/vol) SDS at 95°C and were reprobed to identify in-lane markers by using random-primed labelled marker DNA prepared as described above. Autoradiographs were developed in an automatic film processor (M35A X-OMAT; Eastman Kodak), and computer analysis of DNA patterns was performed with the BioImage Whole Band Analyzer, version 3.1 (Millipore Corporation, Ann Arbor, Mich.), on a Spark 10 workstation. The patterns were compared by using a deviation value of 2%. The similarity (S_{AB}) value was calculated as described elsewhere (22).

RESULTS

Fingerprinting based on IS*6110*. Fingerprint analysis with IS*6110* and *Pvu*II-digested *M. bovis* genomic DNA revealed 16 different fingerprint patterns among 79 bovine isolates (Fig. 1). Seven control isolates originating from deer exhibited multiple bands in two different patterns (patterns 2A [n = 1] and 3C [n = 6]). Three unique patterns representing *M. tuberculosis* controls are also shown in Fig. 1. The resulting patterns were assigned to an IS*6110* class or type represented by a number and a letter. The number represents the number of IS*6110*-containing *Pvu*II genomic fragments detected via Southern blot analysis, and the letter designates each discrete banding pattern. Of the 79 bovine isolates (Texas [n = 17], Mexico [n = 60], Kansas [n = 1], and Mississippi [n = 1]), 64 isolates (81%) exhibited a single IS*6110* copy. Thirty-eight isolates exhibited an IS*6110* copy on a 1.9-kb *Pvu*II restriction fragment (type 1B). Fifteen isolates exhibited a 1.8-kb *Pvu*II restriction fragment (type 1A), seven isolates exhibited a 4.0-kb *Pvu*II restriction fragment (type 1C), and four isolates exhibited a 4.5-kb *Pvu*II restriction fragment (type 1D). In contrast to previous observations, a significant proportion of the bovine isolates (15 of 79) examined in the present study exhibited multiple IS*6110* copies (two to five copies) represented by 11 different patterns. Two different IS*6110* patterns were observed in the deer isolates originating from Montana and New York. Although a majority of bovine isolates in Texas and Mexico harbor a single IS*6110* copy, a significant proportion (19%) contain multiple copies of IS*6110*. Although dendrogram analysis is useful for characterizing closely related organisms, the DR profiles described in the following section proved to be superior (Fig. 2).

Fingerprinting based on DR sequences. Twenty-five distinct DR patterns were observed among the *Alu*I-digested *M. bovis* genomic DNAs from 79 bovine isolates. In addition, seven *M. bovis* isolates originating from deer exhibited two distinct patterns, dr24 (n = 1) and dr23 (n = 6), and each of the three

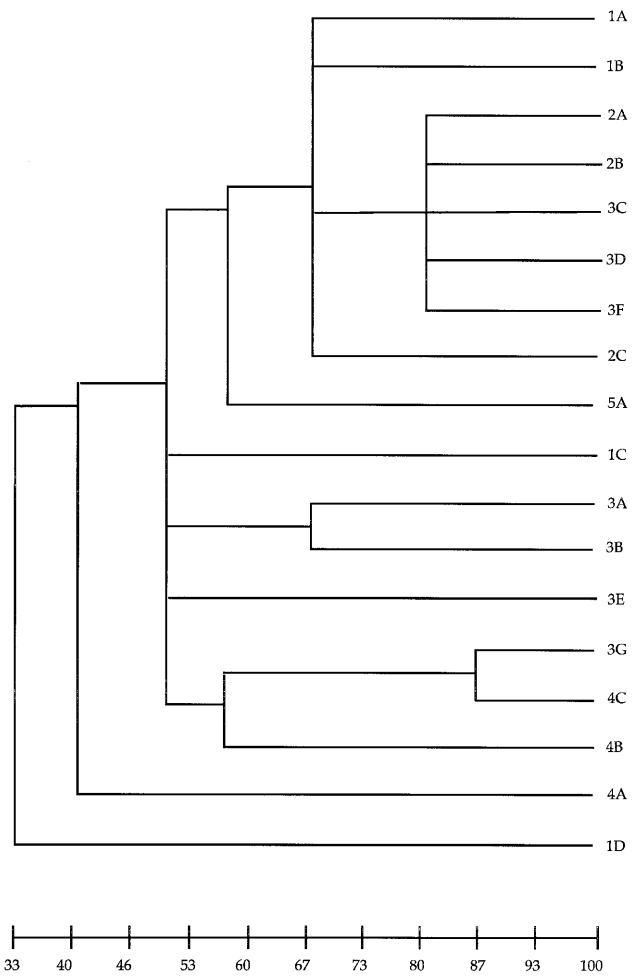

FIG. 2. Dendrogram generated from 18 different IS*6110* profiles representing 86 *M. bovis* isolates. Patterns 2A and 3C represent deer isolates from New York and Montana, respectively. The remaining patterns were observed in isolates from Texas and Mexican cattle.

M. tuberculosis isolates used as controls exhibited unique patterns, dr28 to dr30. Lane maps representing all the DR patterns observed are provided in Fig. 3. The predominant DR type, dr13, was found among 26 isolates originating from both Texas (n = 8) and Mexican (n = 18) cattle. This DR type was shared by three IS*6110* types, IS*6110* type 1B (n = 24), type 3A (n = 1), and type 3B (n = 1). All these isolates share a single IS*6110* copy on a 1.9-kb *Pvu*II restriction fragment. The next predominant DR type, dr18, was found among 13 isolates from Texas (n = 1) and Mexican (n = 12) cattle. Each of these isolates exhibited identical IS*6110* patterns (IS*6110* type 1A). Seven other DR types were shared by at least two isolates originating from the same or different herds, and each of the remaining 16 DR types was represented by a single isolate. In most cases fingerprinting with the DR probe further distinguished among the isolates exhibiting an identical IS*6110* type. For example, IS*6110* type 1B isolates (n = 38) exhibited eight different DR patterns (Table 1).

These data demonstrate differences in the abilities of the IS*6110* and DR probes to categorize *M. bovis* isolates. The DR probe was generally superior to the IS*6110* probe in distinguishing among isolates which often contained a single copy of IS*6110*. On the basis of the individual fingerprint patterns generated by both probes, 79 bovine isolates could be categorized into 30 different RFLP types (Table 1). The deer isolates represented two additional RFLP types (types 19 and 24), and each of the three *M. tuberculosis* isolates represented distinct RFLP types (types 33 to 35).

RFLP analysis of clustered isolates. Among the 30 bovine *M. bovis* RFLP types, only 9 (Table 1) were represented by more than a single isolate (clustered). Each of the remaining 21 types is represented by a single isolate (nonclustered). Clusters were composed of isolates from different regions of Texas and Mexico, as described in Table 2. Although it is not surprising to see varied geographic distributions among nonclustered isolates, the broad distribution observed among clustered isolates may be associated with the movement of cattle between herds. The largest cluster, RFLP type 7, comprised 24 bovine isolates originating from Mexico (n = 17) and Texas (n = 7). The Texas isolates originated from herds in Comanche (n = 2) and Clint (n = 3), 724 km (450 miles) apart. There has been no apparent contact between the Comanche and Clint herds, which contain two different breeds of cattle, Jersey and Holstein, respectively. Thus, it must be assumed that RFLP type 7 is established in Texas. The remaining Texas isolates (n = 2) originated from animals in feedlots in Dumas and San Angelo. The majority of the Mexican isolates (n = 15) were recovered from animals in feedlots dispersed throughout Texas and were traced back to different herds located in geographically disperse regions of Mexico. The remaining isolates (n =

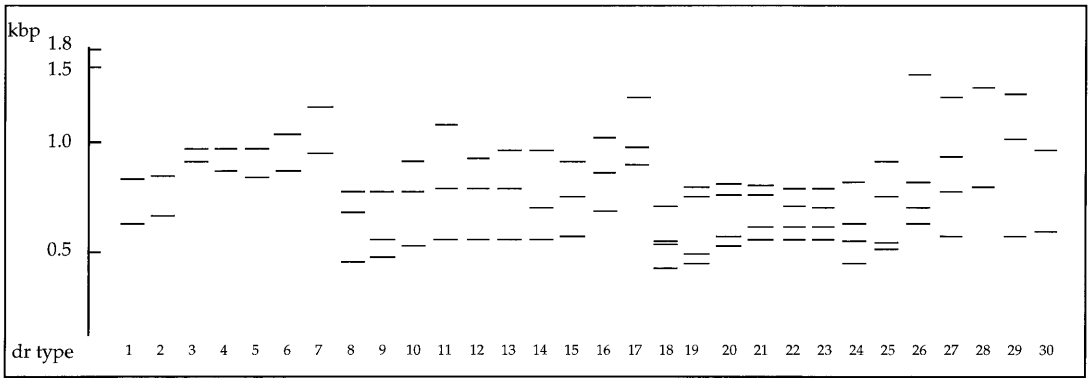

FIG. 3. DR restriction profiles. Lane maps representing 25 distinct DR patterns among bovine isolates along with DR patterns exhibited by deer isolates (dr23, dr24) and *M. tuberculosis* isolates (dr28 to dr30) are shown.

TABLE 1. Geographic distribution of *M. bovis* isolates

RFLP type	IS*6110* type	DR type	No. of isolates from the following location:		
			Mexico	Texas	Other[a]
1	1A	dr8	1		
2	1A	dr9			1^{MS}
3	1A	dr18	12	1	
4	1B	dr1	3		
5	1B	dr11	1		
6	1B	dr12	1		
7	1B	dr13	17	7	
8	1B	dr14	1	1	
9	1B	dr15	1		
10	1B	dr20	5		
11	1B	dr26		1	
12	1C	dr2	1		
13	1C	dr3	3	1	
14	1C	dr5	1		
15	1C	dr17			1^{KS}
16	1D	dr4	2		
17	1D	dr6		1	
18	1D	dr7	1		
19	2A	dr24			1^{NY}
20	2B	dr10	1		
21	2C	dr27	1		
22	3A	dr13		1	
23	3B	dr13	1		
24	3C	dr23			3^{MT},3^{NY}
25	3D	dr1	1		
26	3E	dr16	1		
27	3F	dr19	1		
28	3G	dr21		3	
29	4A	dr25	2		
30	4B	dr22	1		
31	4C	dr21		1	
32	5A	dr19	1		
33	5B	dr28			1^{MTb}
34	6A	dr29			1^{MTb}
35	10A	dr30			1^{MTb}

[a] MT and NY, deer isolates from Montana and New York, respectively; MS and KS, bovine isolates from Mississippi and Kansas, respectively; MTb, *M. tuberculosis* isolates.

2) were recovered from animals in Mexico. These data indicate that isolates belonging to RFLP type 7 are widely distributed in both Mexico and Texas, presumably as a result of active or historical movement of infected or carrier animals (3, 16). At this point, the presence of infected Mexican cattle in Texas feedlots suggests that transmission of isolates of RFLP type 7 was caused in part by cattle importation. The existence of infection in herds not reportedly in contact with Mexican cattle suggests that active foci also exist in Texas. Characterization of these isolates by additional typing methods may help to clarify this situation.

The next largest cluster, RFLP type 3, comprised 13 isolates originating from Mexico ($n = 12$) and Texas ($n = 1$). Mexican bovine isolates ($n = 9$) were recovered from cattle in Texas feedlots which were traced back to herds located in different regions of Mexico. The remaining isolates ($n = 3$) were recovered from animals in Mexico. Although a majority of isolates are widely dispersed, two isolates originated from a single herd in Cuatrocinegas, Coa, Mexico. The lone Texas isolate was recovered from a feedlot animal with an unknown history. On the basis of the observations presented above, it is likely that RFLP type 3 isolates were imported from Mexico. The lone Texas isolate presumably represents an isolate from an animal from Mexico or is the result of transmission from such an animal.

A smaller cluster (RFLP type 28) appeared to be restricted in its geographic distribution to Texas and comprised isolates ($n = 3$) originating from a single herd in Clint. The significant feature of these isolates was the presence of multiple IS*6110* copies within their genomes (IS*6110* type 3G). A related isolate was recovered from an animal in a nearby herd, also in Clint. This isolate exhibited an IS*6110* pattern, type 4C, which shares three IS*6110* bands with IS*6110* type 3G (Fig. 1) and an identical DR pattern (dr21). The difference in IS*6110* profiles suggests the recent duplication of IS*6110*. The restricted distribution and unusual RFLP type suggest the potential for localized transmission among cattle or via wildlife reservoirs.

Most isolates belonging to the remaining clusters originated from Mexican cattle or feedlot animals of Mexican origin or Texas cattle potentially exposed to such animals (Table 2). On the basis of this cluster analysis, it seems likely that the majority of *M. bovis* isolates found in Texas actually originated in Mexico. The low number of cattle originating in Texas and the potential for exposure to Mexican cattle have restricted predictions concerning *M. bovis* isolates originating in Texas.

Nine characterized clusters comprised isolates from within herds and from geographically dispersed herds. The presence of the identical RFLP types within the same herd (Table 2) is consistent with active transmission and confirms previous reports indicating that clustering based on IS*6110* and DR fingerprints is epidemiologically significant (21). The presence of identical RFLP types from different regions of Texas and Mexico is consistent with a broad dispersal of the organisms as a result of active animal movement or dispersal during the past. These data are consistent with the importation of infected Mexican cattle. However, foci of infection within Texas are also apparent.

RFLP analysis of nonclustered isolates. Each of the remaining RFLP types of the bovine isolates is represented by a single isolate. The majority exhibited unique DR patterns, but they had the same limited number of IS*6110* patterns as the clustered isolates. Among these isolates, the DR probe was useful for discrimination according to geographic origin. For example, an isolate from Mississippi of RFLP type 2 shared an IS*6110* pattern (pattern 1A) with Texas and Mexican isolates, but it exhibited a unique DR pattern (dr9). Similarly, a Kansas bovine isolate of RFLP type 15 shared an IS*6110* pattern (pattern 1C) with isolates from Texas and Mexican cattle but exhibited a unique DR pattern (dr17). The observed similarity in IS*6110* and DR patterns of deer isolates from New York and Montana suggest a close relationship, but the similarity could not be confirmed epidemiologically.

Interestingly, few isolates shared DR patterns and exhibited distinguishing IS*6110* patterns. This is surprising since changes in both patterns are caused by independent recombination events which overlap at the DR locus (11). The effect of multiple IS*6110* insertions may be examined by comparing such isolates. One example is the isolates with RFLP type 22 ($n = 1$), RFLP type 23 ($n = 1$), and RFLP type 7 ($n = 24$), which shared a common DR type (dr13) but which exhibited different IS*6110* patterns (patterns 3A, 3B, and 1B respectively). Increased levels of IS*6110* insertion may explain the differences in the distributions of these isolates. The recent addition of two copies of IS*6110* to types 22 and 23 may be directly responsible for their limited distribution as a result of an attenuated phenotype and confirms the predicted stability of the DR patterns. If this is correct, it does not extend to *M. tuberculosis* isolates,

TABLE 2. Geographic distributions of clustered *M. bovis* isolates

Origin	No. of isolates of the following RFLP type:									
	3	4	7	8	10	13	16	24	28	29
Mexico[a]										
Aguascalientes, AGS	1		1							
Cuatro Cinegas, COA	2									
Monclova, COA	1									
Saltillo, COA	1		1		1					
Mexico City, MEX	2	1	5	1	3	1	1			2
UNAM, Mexico City, MEX[b]	3	2	2							
Monterrey, N.L.			1							
Pesquesia, N.L.			1							
Valecillo, N.L.			1		1	1				
Villaldamo, N.L.							1			
N. Durango, DUR	2		3							
Hermosillo, SON						1				
San Luis Potosi, SLP			1							
Reynosa, TAM			1							
Texas										
Clint			3						3	
Comanche			2							
Fabens						1				
Dalhart[c]				1						
Dumas[c]			1							
Plainview[c]	1									
San Angelo[c]			1							
Other										
Hardin, Mont.								2		
Phillipsburg, Mont.								2		
Addison, N.Y.								2		
Total	13	3	24	2	5	4	2	6	3	2

[a] Mexican states: AGS, Aguas Calientes; COA, Coahuila; N.L., Nuevo Leon; DUR, Durango; TAM, Tamualipas; SLP, San Luis Potosi; MEX, Mexico.
[b] UNAM, Universidad Nacional Autonoma de Mexico.
[c] Feedlot cattle.

which have many more IS*6110* copies with no apparent effect on virulence (12).

DISCUSSION

Epidemiological studies with molecular markers were performed in the past in New Zealand, Australia, The Netherlands, the United Kingdom, Spain, and Sweden (3, 10, 16, 17, 21). No such detailed study has been performed in the United States because of the low incidence of disease. The recent rise in the number of cases of bovine tuberculosis, especially in Texas, has caused alarm among beef and dairy producers interested in identifying the source(s) of infection. In the present study, IS*6110* and DR probes were used to gather information on the number of *M. bovis* strains isolated from cattle within Texas and Mexico. Our findings indicate that isolates of several RFLP types (determined on the basis of their DR and IS*6110* fingerprints) are found in Texas and Mexican cattle, suggesting multiple foci of infection.

In the present study isolates originating from deer and cattle were shown to contain multiple IS*6110* copies. The evidence reported here indicates a limited distribution of such organisms, and it has been suggested by us and others that such organisms may be attenuated for virulence in cattle (10). A wildlife reservoir in which these isolates retain their virulence may represent a source of infection. In the past only isolates from antelopes, oryxes, monkeys, seals, deer, or goats (10, 17, 21) have been shown to harbor multiple IS*6110* copies. Potential reservoirs of *M. bovis* in Texas and Mexico include deer, feral pigs, and peccaries. Recent isolations of *M. bovis* from deer in Texas have confirmed this suspicion. Fingerprinting Texas deer isolates and surveying herds for those infected with isolates with multiple IS*6110* copies would provide the necessary epidemiological information to establish an association between a particular RFLP type and the severity of the lesions.

A computer database of different fingerprints will be maintained. This will be used to display the results geographically to uncover the epidemiological basis for the distributions of distinct RFLP types. Other molecular typing methods, including fingerprinting with the pTBN12 probe (14) and spoligotyping (23), will be used in an effort to further distinguish *M. bovis* isolates.

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