JOURNAL OF CLINICAL MICROBIOLOGY, July 1996, p. 1617–1621 Vol. 34, No. 7
0095-1137/96/$04.00+0

Molecular Characterization of *Mycobacterium paratuberculosis* Isolates from Sheep, Goats, and Cattle by Hybridization with a DNA Probe to Insertion Element IS*900*

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Received 14 August 1995/Returned for modification 17 October 1995/Accepted 4 March 1996

Mycobactin J-dependent mycobacterial isolates from sheep, goat, and cattle herds with Johne's disease in Morocco, South Africa, the United States, and Germany were tested for the repetitive insertion sequence IS*900* of *Mycobacterium paratuberculosis* by PCR. The IS*900* PCR target sequence was detected in 90 of 93 fecal culture isolates tested (96.8%). Restriction fragment length polymorphisms (RFLPs) and in vitro growth characteristics were studied in 46 of the IS*900*-positive isolates and in two bovine vaccine strains of *M. paratuberculosis*. Five different RFLP types were identified in *Pvu*II digests of genomic DNA by Southern hybridization with a DNA probe specific for IS*900*. All isolates of *M. paratuberculosis* could be classified into two major clusters by their growth rates as well as the relatedness of their *Pvu*II-RFLP hybridization patterns. All of the sheep isolates were classified into cluster I (extremely slow growth), while all cattle and goat isolates were members of cluster II (moderately slow growth). Different *Pvu*II-RFLP patterns were detected in different sheep flocks from Morocco and South Africa. Our results demonstrate that genetically and phenotypically different strains of *M. paratuberculosis* were present in ruminant populations. The strains from sheep in Morocco and South Africa tested in the study appeared to belong to a unique group of *M. paratuberculosis* strains that might have adapted to this host species. The presence of several genetically distinct strains in different sheep flocks suggested that analysis of IS*900*-specific RFLP patterns may provide a useful tool for the epidemiologic investigation of ovine paratuberculosis outbreaks.

Paratuberculosis or Johne's disease (JD) is a chronic infectious disease that affects all categories of domestic and wild ruminants including cattle, sheep, goats, camels, buffaloes, and farmed deer (20, 26, 33, 38). JD is known to occur worldwide and is responsible for important economic losses (8, 41). The causative agent, *Mycobacterium paratuberculosis*, is a slowly growing, acid-fast microorganism that, unlike other cultivable mycobacteria, requires exogenous supplementation with ferric mycobactin J for growth in primary culture (8). In humans, *M. paratuberculosis* is considered to be a potential etiologic agent in cases of Crohn's disease (22).

Although the etiology of paratuberculosis has been known for decades, several questions concerning the pathogenesis and epidemiology of the disease are still open. Research has long been hampered by the lack of sensitive methods for the systematic differentiation of *M. paratuberculosis* strains. Phenotypic and genetic properties, e.g., colony morphology and pigmentation, biochemical reactions, antigen and cellular fatty acid compositions, and restriction endonuclease fragment patterns, which are reliable criteria for subtyping many other microorganisms, were inadequate for subtyping *M. paratuberculosis* strains (5–7, 9, 29, 39, 41). The detection of IS*900*, a repetitive DNA insertion element, in an isolate of *M. paratuberculosis* from a patient with Crohn's disease represented a milestone in the search for a genetic marker for this pathogen. IS*900* has a unique nucleotide sequence which can be specifically detected by hybridization or PCR techniques (10, 12, 17, 25, 37). Sequences of IS*900* proved to be highly sensitive and specific markers of *M. paratuberculosis* among other slowly growing, acid-fast bacteria, because IS*900* has been detected in all reference and vaccine strains as well as in field isolates of *M. paratuberculosis* from several hosts but never in other bacterial species (1, 10, 17, 18, 24, 25, 40). Furthermore, the use of DNA fingerprinting techniques based on IS*900* as the target sequence has been the most successful approach to the molecular characterization of *M. paratuberculosis* strains. Complex banding patterns are generated when Southern blots of restricted genomic DNA are hybridized with a probe to IS*900* because *M. paratuberculosis* strains harbor multiple copies of IS*900* at several sites of their chromosomes (23). This restriction fragment length polymorphism (RFLP) demonstrated a considerable degree of genetic variability between several *M. paratuberculosis* isolates, thus making the identification of individual strains and clonal lineages possible (11, 12, 16, 36, 40).

Recent findings have suggested that different growth rates on primary culture and IS*900*-specific RFLP separate *M. paratuberculosis* strains into two major groups. One of these major groups consists of strains which have exclusively been recovered from sheep and goats, while the other one comprises strains which are commonly associated with bovine paratuberculosis but which can also be detected in sheep and goats (11, 16, 36, 40). However, this concept of two major groups with different host preferences and geographic distributions is still based on data obtained from studies of a rather limited number of *M. paratuberculosis* strains and from only a few countries where animals are known to be affected by paratuberculosis.

In the present study, mycobactin J-dependent mycobacteria isolated from three sheep herds with JD in Morocco were

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characterized and compared with similar strains from sheep, cattle, and goats in Morocco, South Africa, Germany, and the United States. Fecal culture isolates were tested for IS*900* sequences by PCR and for growth characteristics in vitro. Genomic variations between *M. paratuberculosis* isolates of different animal or geographic origins were demonstrated by a DNA fingerprinting procedure with a digoxigenin (DIG)-labelled DNA probe specific for IS*900*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A total of 93 mycobacterial field isolates was examined in the study. All of the strains had been cultured from fecal samples by standard procedures and were maintained aerobically at 37°C on Loewenstein-Jensen solid medium supplemented with mycobactin J (Rhone Merieux, Laupheim, Germany). Isolates were presumptively identified as *M. paratuberculosis* on the basis of slow growth, mycobactin J dependency, acid fastness (by Ziehl-Neelsen staining), and colony morphology (8). The strains obtained in Morocco were isolated during a survey of three farms located 85 km north (farms M1 and M2) and 15 km south (farm M3) of the city of Rabat. A frequent exchange of some animals between farms M1 and M2 was reported. On farm M1, 17 presumptive *M. paratuberculosis* strains were isolated from 51 sheep by fecal culture methods, 23 strains were isolated from 80 sheep on farm M2, and 14 strains were isolated from 49 sheep on farm M3. On farm M1, one strain was also occasionally isolated from a cow. All animals tested were suspected of suffering from paratuberculosis because they exhibited slow, progressive weight loss and in some cases had diarrhea nonresponsive to anthelmintic or antibiotic treatment. Isolation of *M. paratuberculosis* strains from sheep on farm M1 and corresponding clinical and histopathological findings have been published recently (3). The Moroccan strains were compared with a collection of similar strains which originated in South Africa (2 strains from sheep on 1 farm), Germany (25 strains from goats on 1 farm and 10 strains from cattle on 10 farms), and the United States (one strain from cattle). German strains had been isolated at the Institute for Hygiene and Infectious Diseases of Animals, Giessen, Germany. The South African strains were a kind gift of the Veterinary Institute in Onderstepoort, South Africa. The U.S. strain was obtained from the Department of Microbiology, Iowa State University, Ames. Bovine *M. paratuberculosis* vaccine strains 316F (Rhone Merieux) and EII (Central Veterinary Laboratory, Weybridge, United Kingdom) were used as reference strains.

DNA isolation. Genomic DNA was extracted from the mycobacterial strains by the method described by Whipple et al. (41). Bacterial cells were harvested (100 mg [wet weight]), washed in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), and incubated in TE buffer containing lipase (final concentration, 16,000 U/ml) for 2 h at 37°C. After the addition of lysozyme (5 mg/ml) and incubation for 2 h at 37°C, samples were supplemented with sodium dodecyl sulfate (SDS; 10 mg/ml) and proteinase K (2 mg/ml), and incubation at 37°C was continued for 16 h. Subsequently, samples were gently mixed with 0.4 volume of 5 M potassium acetate, and the mixture was placed on ice for 10 min. After centrifugation, DNA was purified from the supernatant by repeated phenol-chloroform-isoamyl alcohol (25:24:1; vol/vol/vol) extraction and was precipitated with ethanol. The precipitated DNA was dissolved in sterile twice distilled water.

PCR. Enzymatic amplification of DNA was performed by a standard PCR technique described previously (27). PCR mixtures of 20 μl contained 1× Tfl buffer (Biozym, Hameln, Germany), 0.16 mM (each) dATP, dGTP, dCTP, and dTTP, 1.0 μM (each) primer, 0.1 U of *Thermus flavus* thermostable DNA polymerase (Biozym), and 0.2 μl of purified genomic DNA solution. PCR primers MP3 (5′-CTGGCTACCAAACTCCCGA-3′) and MP4 (5′-GAACTCAG CGCCCAGGAT-3′) were derived from the published DNA sequence of IS*900* and flank a 314-bp internal segment of IS*900* (Genbank/EMBL accession number X16293) (17). PCR was performed by using 30 cycles of denaturation (94°C, 1 min), primer annealing (65°C, 2 min), and synthesis (72°C, 3 min) on a programmable thermal cycler (Perkin-Elmer Cetus, Langen, Germany). Amplicons were detected by visualization on agarose gels stained with ethidium bromide.

RFLP analysis. Approximately 4 μg of purified mycobacterial DNA was digested with 8 U of *Pvu*II (Pharmacia, Freiburg, Germany) at 37°C for 3 h. DNA fragments were separated by electrophoresis through gels (11 by 18 cm) of 0.8% agarose (Tris-borate-EDTA buffer, 25 V, 18 h). Subsequently, the DNA fragments were exposed to UV illumination (302 nm, 5 min) and transferred onto a positively charged nylon membrane (no. 1417240; Boehringer, Mannheim, Germany) with 0.4 M NaOH by standard capillary blotting procedures (28). The blots were neutralized with 1.5 M NaCl–0.5 M Tris-Cl–1 mM EDTA (pH 8.0), and DNA was fixed by UV illumination (300 mJ/cm^2, 3 min). For hybridization, an internal amplicon from the IS*900* of *M. paratuberculosis* vaccine strain 316F was used as the probe. The probe was synthesized and labelled with DIG by the PCR method described above, except that dTTP was reduced to 0.125 mM dTTP and 0.035 mM DIG-11-dUTP (Boehringer) was added to the PCR mixture. DNA blots were prehybridized in hybridization buffer (50% [vol/vol] formamide, 5× SSC [75 mM sodium citrate plus 750 mM NaCl; pH 7.0], 2% [wt/vol] blocking reagent, 0.1% [wt/vol] *N*-lauroylsarcosine, 0.02% [wt/vol] SDS) at 42°C for 3 h.

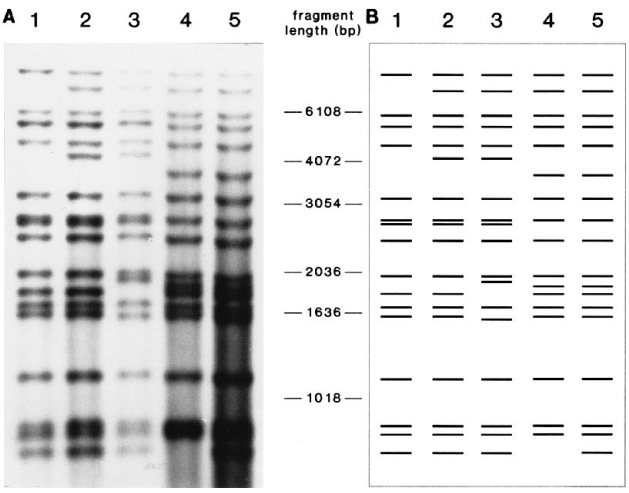

FIG. 1. Autoluminogram (A) and normalized schematic representation (B) of Southern blot hybridization patterns of *M. paratuberculosis* fecal culture isolates from sheep, goats, and cattle. Purified genomic DNA was digested with *Pvu*II, separated by agarose gel electrophoresis, blotted onto a nylon membrane, and hybridized with a DIG-labelled DNA probe specific for insertion element IS*900*. DNA was purified from representative isolates yielding *Pvu*II-RFLP types 1 to 5 (lanes 1 to 5, respectively).

Hybridization was carried out at 42°C for 16 h with fresh hybridization buffer (0.1 ml/cm^2) containing the denatured probe (1 ng/ml). Then, the blots were washed twice at 42°C for 20 min in 0.5× SSC containing 36% (wt/vol) urea and 0.4% (wt/vol) SDS and twice at room temperature for 5 min with 2× SSC. DNA–DIG-DNA hybrids were visualized by autoluminography with the commercially available DIG luminescent detection kit (Boehringer), as recommended by the manufacturer.

RESULTS

PCR results and comparison of in vitro growth rates. All 93 fecal isolates were mycobactin J dependent on primary culture. In each case smears of these cultures on glass slides showed only clumps of short red bacilli after Ziehl-Neelsen staining. However, two different phenotypes were observed on the basis of the bacterial growth rate. One phenotype was characterized by colonies macroscopically not visible before 16 weeks of incubation (slow growth). Upon the first subcultivation these strains retained the very slow growth rate of the parent culture. Colonies of the other phenotype appeared significantly faster and were macroscopically visible after 6 to 12 weeks of incubation (fast growth). Each of these growth phenotypes was strictly associated with particular host species. Slow growers were only observed in cultures of fecal samples from sheep, while isolates in cultures of fecal samples from goats and cattle as well as reference strains from cattle exclusively yielded colonies of the fast grower phenotype. The growth rate did not discriminate between strains from different herds or different geographic areas.

The IS*900* PCR target sequence was detected in 90 of the 93 fecal culture isolates (96.8%) and in both *M. paratuberculosis* vaccine strains after PCR amplification. Three mycobactin J-dependent isolates (one per host species examined) were IS*900* negative by PCR, and IS*900*-related sequences could not be detected in these strains by Southern blot hybridization.

Differentiation of *M. paratuberculosis* fecal culture isolates by analysis of *Pvu*II-RFLPs. Purified genomic DNA from all cattle strains and several randomly selected strains of *M. paratuberculosis* from Moroccan sheep and German goats was digested with *Pvu*II and analyzed by DNA fingerprinting by using a probe specific for IS*900*. As presented in Fig. 1A (autolumi-

TABLE 1. Growth rates and *Pvu*II-RFLP types of *M. paratuberculosis* fecal culture isolates of ovine, caprine, and bovine origins

Host species	Country	Farm	No. of isolates tested	Properties of isolates	
				Growth rate	RFLP type
Sheep	Morocco	M1	8	Slow	1
		M2	12	Slow	1
		M3	6	Slow	2
	South Africa	S1	2	Slow	3
Goat	Germany	G1	7	Fast	4
Cattle	Morocco	M1	1	Fast	4
	Germany	G2	1	Fast	4
		G3	1	Fast	4
		G4	1	Fast	4
		G5	1	Fast	4
		G6	1	Fast	4
		G7	1	Fast	4
		G8	1	Fast	4
		G9	1	Fast	4
		G10	1	Fast	5
	United States	U1	1	Fast	4

nogram) and Fig. 1B (corresponding normalized schematic representation), a total of five different hybridization patterns could be defined on the basis of the numbers and sizes of the IS*900*-positive DNA fragments. The patterns were designated 1, 2, 3, 4, and 5. The numbers of hybridized bands varied from 16 to 18 per pattern. Fragment lengths were approximately 750 to 8,800 bp. Overall, 22 different IS*900*-positive DNA fragments were observed in all strains tested. Differences between the *Pvu*II-RFLP patterns were restricted to 10 fragments. Twelve fragments were common in all patterns.

Association between *Pvu*II-RFLP types and origins of *M. paratuberculosis* fecal culture isolates. As shown in Table 1 and Fig. 2, the *Pvu*II-RFLP types of all IS*900*-positive isolates (n = 46) were strictly associated with a particular host species. *Pvu*II-RFLP types 1, 2, and 3 were exclusively detected in sheep strains, while types 4 and 5 were only seen in cattle and goat isolates. Only caprine and bovine isolates shared the same *Pvu*II-RFLP type. *Pvu*II-RFLP type 4 was predominant in goat and cattle isolates (17 of 18 field isolates). All isolates with *Pvu*II-RFLP type 1, 2, or 3 were classified as slow growers, while all isolates with *Pvu*II-RFLP type 4 or 5 exhibited the fast growth phenotype.

The sheep-associated *Pvu*II-RFLP types correlated with a particular herd or geographic area of origin. All *M. paratuberculosis* isolates from cultures of fecal samples from sheep on the same farm had identical *Pvu*II-RFLP patterns. Strains isolated from animals on farms M1 and M2 were indistinguishable by their *Pvu*II-RFLP patterns (type 1). Isolates from animals on farm M3 (type 2) and those from a South African sheep flock (type 3) each formed a distinct group. In contrast, cattle and goat isolates showed almost no variation, although they were isolated in separate areas of the world. Different *Pvu*II-RFLP types were observed only among bovine *M. paratuberculosis* fecal culture isolates from Germany (*Pvu*II-RFLP types 4 and 5). Both bovine *M. paratuberculosis* reference strains (strains 316F and EII) also yielded *Pvu*II-RFLP type 4.

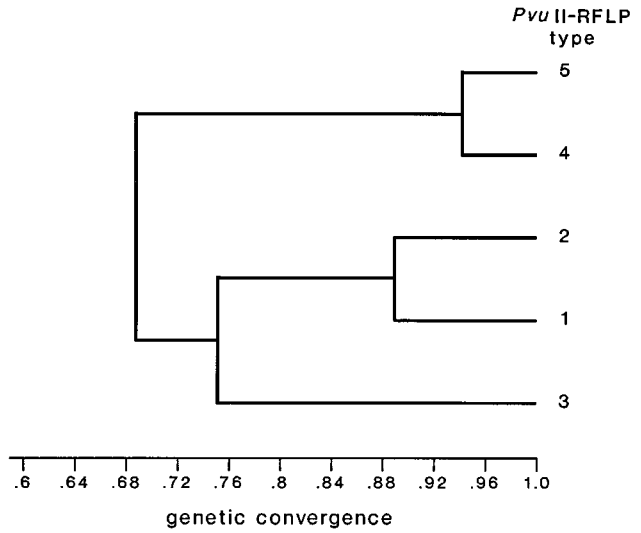

FIG. 2. Dendrogram of DNA convergence generated from banding patterns of *Pvu*II-RFLP types 1 to 5 of *M. paratuberculosis* fecal culture isolates by the unweighted paired group method with arithmetic averages (31).

The single *M. paratuberculosis* strain obtained from a cow on farm M1 was different from the eight isolates from sheep on this farm (slow growth, *Pvu*II-RFLP type 1), but its growth rate as well as its *Pvu*II-RFLP pattern matched exactly those of strains isolated from cattle in Germany and the United States.

The association of *Pvu*II-RFLP patterns with host species and herd or country of isolation was also demonstrated when the DNA divergence of the five different *Pvu*II-RFLP patterns was estimated by distance matrix calculation by the unweighted paired group method with arithmetic averages of Sneath and Sokal (31). The dendrogram in Fig. 2 shows two major clusters of *M. paratuberculosis* isolates. Cluster I is represented by all of the extremely slowly growing sheep strains (*Pvu*II-RFLP types 1, 2, and 3), and cluster II is represented by all of the moderately slowly growing (fast) cattle and goat strains (*Pvu*II-RFLP types 4 and 5). The genetic relationship between these two clusters was 69.5%. Regionality was observed in ovine *Pvu*II-RFLP patterns, since *Pvu*II-RFLP types from Moroccan isolates (types 1 and 2) were more closely related to each other than to the South African *Pvu*II-RFLP type (89 versus 75%). The closest relationship (approximately 95%) was exhibited by *Pvu*II-RFLP patterns 4 (cattle and goat strains) and 5 (cattle strains), which differed by only one IS*900* band.

DISCUSSION

The data obtained in the present study are consistent with the hypothesis of the existence of at least two major groups of *M. paratuberculosis* strains, each having its own phenotypic and genetic characteristics and each showing genetic heterogeneity to some extent. In this report, all of the *M. paratuberculosis* fecal culture isolates and reference strains were grouped into two major clusters according to the growth rate in vitro as well as to the relatedness of their hybridization patterns. The *Pvu*II-RFLP-based major classification exactly matched with the differentiation into slowly growing or fast-growing strains. Accordingly, each *Pvu*II-RFLP pattern was associated only with slow or fast growth, and hybridization patterns were more closely related to each other for strains belonging to the same growth phenotype. Remarkably, this dual scheme of classification is consistent with the results obtained for *M. paratubercu-*

losis strains isolated from very distant areas like Norway, the Faroe Islands, Canada, Australia, New Zealand, and South Africa (11, 12, 16, 36).

The classification of our fecal culture isolates into either the fast or the slow group of *M. paratuberculosis* strains was exactly correlated with their origins in cattle and goats or in sheep, respectively. Even in the mixed animal farming situation on farm M1 in Morocco, a bovine strain was a fast grower, with the most prevalent *Pvu*II-RFLP pattern of this group, while all isolates from sheep on that farm were typical members of the slowly growing group of strains. This strict association with a particular host species may represent the different host species preferences of both major groups of *M. paratuberculosis* strains that have been suggested previously on the basis of epidemiological data (2, 30, 34) as well as on the basis of molecular microbiological analysis of isolates (11, 16, 36). Difficulties with the primary cultivation of *M. paratuberculosis* isolates from sheep has long been recognized to be specific for isolates from this animal species (11, 12, 19, 21). In the present study as well as in reports of other studies, extremely low rates of replication were always correlated with particular *Pvu*II-RFLP patterns, indicating that the reduced in vitro growth rate is a very specific property of a distinct group of *M. paratuberculosis* strains rather than of the host species or of the sample material investigated (11). According to the different distributions among farm animal species, Collins and colleagues (11, 12) designated the group of strains growing easily on primary cultivation the "cattle group," while ovine field strains with extremely poor growth were termed the "sheep group." In agreement with our results, the *M. paratuberculosis* vaccine strain 316F was a typical cattle group strain (11, 12, 16). However, members of the sheep group of strains have also occasionally been isolated from goats, and strains with characteristics of the cattle group were detected in some sheep as well (11, 40). These findings indicate that both major groups of *M. paratuberculosis* strains prefer certain animal species rather than are truly host species restricted, as suggested by our data. Furthermore, our data support but cannot definitively prove this concept of host species preference, because almost all isolates of *M. paratuberculosis* studied here were different from each other in both parameters: the animal species and the herd (or location) of origin. The possibility that the local predominance of different strains of *M. paratuberculosis* has biased the association observed between specific strains and animal species cannot be excluded. Host species preference seems to be a real phenomenon among *M. paratuberculosis* isolates; however, it is a hypothesis based on accidental observations rather than on the results of systematic research. The number of strains which have been characterized by molecular microbiological techniques is still small, and these strains were usually not from truly mixed populations of different ruminant species. Further studies with genetically well defined strains should help to precisely assess the variations in host species adaptation among *M. paratuberculosis* strains. They should also help to elucidate the role of sheep in the transmission of these pathogens to other animal species and humans.

The discrimination of the sheep strains into three distinct *Pvu*II-RFLP types was clearly associated with the herd or the geographic area of their origin, indicating that ovine strains are heterogeneously distributed and that some strains may be present only regionally. We presume that this heterogeneous distribution reflects the evolution of different clonal lineages of *M. paratuberculosis* in separate sheep populations. Previous reports support this conclusion (11, 36). However, at least some ovine strains seem to be distributed internationally, as indicated by the detection of sheep strains genetically indistinguishable from each other in South Africa and Canada (16).

It is remarkable that all ovine *M. paratuberculosis* strains isolated on the same farm or from sheep that had had contact with sheep on this farm yielded identical IS*900*-specific banding patterns, while isolates from sheep herds with no contact to each other always exhibited different IS*900* profiles. Insertion sequences like IS*900*, which are present in multiple copies in a single bacterial chromosome, are likely to undergo rearrangements and recombination more rapidly than less mobile parts of the genome. Furthermore, DNA mutations can cause the loss or acquisition of specific restriction sites and may also contribute to the rapid generation of genetic heterogeneity. The detection of single RFLP types within specific sheep herds suggests that JD in each of these herds was due to the epidemic infection with a single strain of *M. paratuberculosis*. Possibly, this strain has been introduced into the herd by a single source and was transmitted to the succeeding generation.

Surprisingly, almost all cattle and goat fecal culture isolates in the present study had identical *Pvu*II-RFLP patterns, although they were from different farms and in some instances were even from different continents. In contrast to these results, other investigators have demonstrated various RFLPs among *M. paratuberculosis* isolates from cattle in New Zealand, Australia, and the United States (11, 40). Most of these RFLP variations were only detected if the digestion of genomic DNA was performed with endonucleases other than *Pvu*II, which was used in the present study. From these findings, *Pvu*II does not seem to be the enzyme with the most discriminatory potential, particularly in the cattle group of strains. Probably, the cattle and goat isolates of the present study (*Pvu*II-RFLP types 4 and 5) could be further differentiated if other endonucleases would be used alternatively or additionally.

Three acid-fast fecal culture isolates of the present study were mycobactin J dependent and needed at least 7 weeks of culture for visible growth in vitro, but they did not harbor any copy of IS*900*, as shown by negative PCR and hybridization results. The lack of IS*900* indicated that these isolates were not *M. paratuberculosis*. Dependence on exogenous mycobactin J for growth in vitro is still widely used as a specific criterion for *M. paratuberculosis*, but it has also been demonstrated in several other members of the *M. avium* complex, particularly the wood pigeon bacillus (14, 35, 36). Mycobacteria of the *M. avium* complex other than *M. paratuberculosis* have often been detected in domestic animals including ruminants (1, 14, 36). These data and the experimental data presented here would suggest that our IS*900*-negative isolates were most probably members of the *M. avium* complex. However, the possibility that they represented a different group of slowly growing mycobacteria, as has been suggested for similar mycobacteria isolated from goats with JD in Norway (36), cannot be definitively excluded. Each of the IS*900*-negative isolates in the present study was obtained from herds with cases of clinical JD. However, the pathogenic potential of these isolates remains uncertain because they were always coisolated with strains of *M. paratuberculosis* from the same herd. Nevertheless, several members of the *M. avium* complex can cause systemic infection in avian hosts as well as in mammals. Calves experimentally infected with the wood pigeon bacillus, another IS*900*-negative member of the *M. avium* complex, even developed clinical signs of JD (15). Recently, several modern molecular microbiological techniques based on IS*900* as the target sequence have been developed and have been suggested to be highly useful for the etiological diagnosis of JD in ruminant farm animals (4, 13, 25, 32, 37). These techniques would be of

limited diagnostic value if IS*900*-negative mycobacteria have any significance in the pathogenesis and epidemiology of JD.

In conclusion, the finding of five distinct *Pvu*II-RFLP types in a relatively small number of *M. paratuberculosis* isolates underlines previous reports that in spite of great similarity between isolates of *M. paratuberculosis*, genetically different strains exist in ruminant populations (11, 12, 16, 36, 40). The heterogeneous distribution of genetically different ovine strains of *M. paratuberculosis* suggests that the DNA fingerprinting method based on IS*900* as a marker provides a valuable tool in epidemiological studies of paratuberculosis in sheep.

ACKNOWLEDGMENTS

We thank L. H. Wieler and Michael Schmidt for suggestions on the manuscript.

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