

RESEARCH COMMUNICATION

**CHARACTERIZATION OF OVINE STRAINS OF *MYCOBACTERIUM PARATUBERCULOSIS* BY RESTRICTION ENDONUCLEASE ANALYSIS AND DNA HYBRIDIZATION**

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ABSTRACT

DE LISLE, G. W., COLLINS, D. M. & HUCHZERMAYER, H. F. A. K., 1992. Characterization of ovine strains of *Mycobacterium paratuberculosis* by restriction endonuclease analysis and DNA hybridization. *Onderstepoort Journal of Veterinary Research*, 59, 163–165 (1992)

Restriction endonuclease analysis and DNA hybridization revealed five ovine strains of *Mycobacterium paratuberculosis* from South Africa had identical DNA patterns to an ovine strain from Canada. Genetically this strain type has features in common with the two major groups of *M. paratuberculosis*.

INTRODUCTION

Johne's disease is a chronic wasting enteropathy of domestic ruminants, including sheep. Early reports of Johne's disease stated that *M. paratuberculosis* isolated from sheep may differ from those obtained from cattle (Taylor, 1945). The principal difference observed between sheep and cattle strains was that the former were more difficult to grow on primary culture. In addition, a pigmented strain of *M. paratuberculosis* appeared to have a host preference for sheep (Taylor, 1951). However, the evidence from experimental infections did not support the hypothesis of host adapted strains of *M. paratuberculosis*. The strains from Iceland and Scotland, that were believed to be sheep adapted, produced clinical Johne's disease in cattle under experimental conditions (Taylor, 1953). Similarly, strains from cattle have been used to experimentally produce Johne's disease in sheep (Kluge, Merkal, Monlux, Larsen, Kopecky, Ramsey & Lehmann, 1968). The results of these experiments may be misleading because the majority of the experimental infections have been established with very large numbers of organisms.

Recent investigations have demonstrated that DNA restriction endonuclease analysis is a useful method for characterizing strains of *M. paratuberculosis*. Initial studies indicated that there was very little genetic diversity among bovine strains of *M. paratuberculosis* (Collins & De Lisle, 1986; Whipple, Kapke & Andrews, 1989). A subsequent study which used both restriction endonuclease analysis and DNA hybridization revealed the presence of two major groups of *M. paratuberculosis* (Collins, Gabric & De Lisle, 1990). One of the groups consisted principally of strains from cattle, with a smaller number of representatives from sheep and goats. The other group was comprised only of strains from sheep and goats. In this study we characterized five South African sheep

strains by restriction endonuclease analysis and DNA hybridization.

MATERIALS AND METHODS

The five isolates of *M. paratuberculosis* characterized in this study were isolated from the small intestine of sheep with clinical Johne's disease. These animals came from Nooitgedacht Agricultural Research Station at Ermelo in Eastern Transvaal. Isolation procedures were as described in Laboratory Methods in veterinary Mycobacteriology, Ames, USA (1974), except that 0,75 % hexadecylpyridinium chloride was used instead of 0,1 % benzalkonium chloride for decontamination. On primary isolation small numbers of colonies (range 3–51 colonies/sheep) of *M. paratuberculosis* were obtained even though the tissues cultured contained very large numbers of acid fast bacteria as demonstrated by microscopic examination of Ziehl-Neelsen stained smears.

The methods for the DNA extraction procedure, restriction digests and DNA hybridization have been described previously (Collins, Gabric & De Lisle, 1990). The probe used for the hybridizations was a 0,22 kb fragment of a repetitive DNA element isolated from the type strain of *M. paratuberculosis* (Collins, Gabric & De Lisle, 1989).

RESULTS

The five strains of *M. paratuberculosis* were identical by restriction endonuclease analysis and DNA hybridization. The strains were also identical to a strain of *M. paratuberculosis* isolated from a sheep in Canada (Collins, Gabric & De Lisle, 1990). The restriction endonuclease pattern of the South African strains appears to be more similar to that of the sheep group than the cattle group of *M. paratuberculosis* (Fig. 1), while the hybridization pattern is intermediate between that of the two major groups (Fig. 2).

DISCUSSION

Characterization of five ovine strains of *M. paratuberculosis* from South Africa revealed that they had identical DNA restriction endonuclease and hybridization patterns to those previously seen only in a

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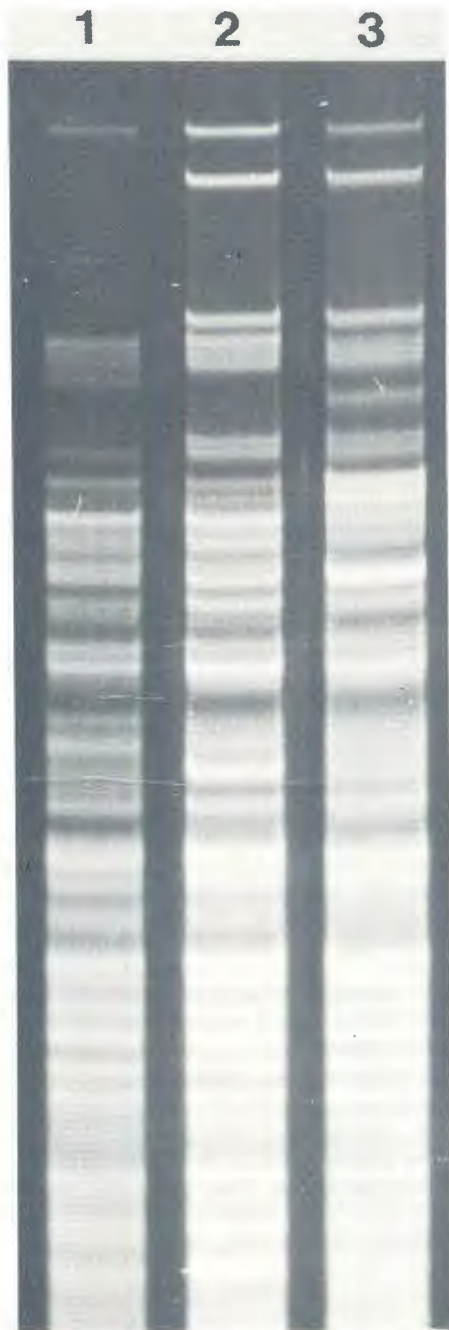


FIG. 1 DNA restriction fragment patterns after *Bst*EI digestion of *M. paratuberculosis* strains isolated from: 1. cattle, 2. South African sheep, 3. New Zealand sheep

single strain from a Canadian sheep (Collins, Gabric & De Lisle, 1990). These strains are of considerable interest because there are similarities between their DNA restriction and hybridization patterns and the patterns of the two distinct groups into which *M. paratuberculosis* has been characterized. One of these groups which principally consists of strains of *M. paratuberculosis* from cattle, has a wide international distribution (Collins, Gabric & De Lisle, 1990; Whipple, Kapke & Vary, 1990). A characteristic of this group is that they are relatively easy to grow on

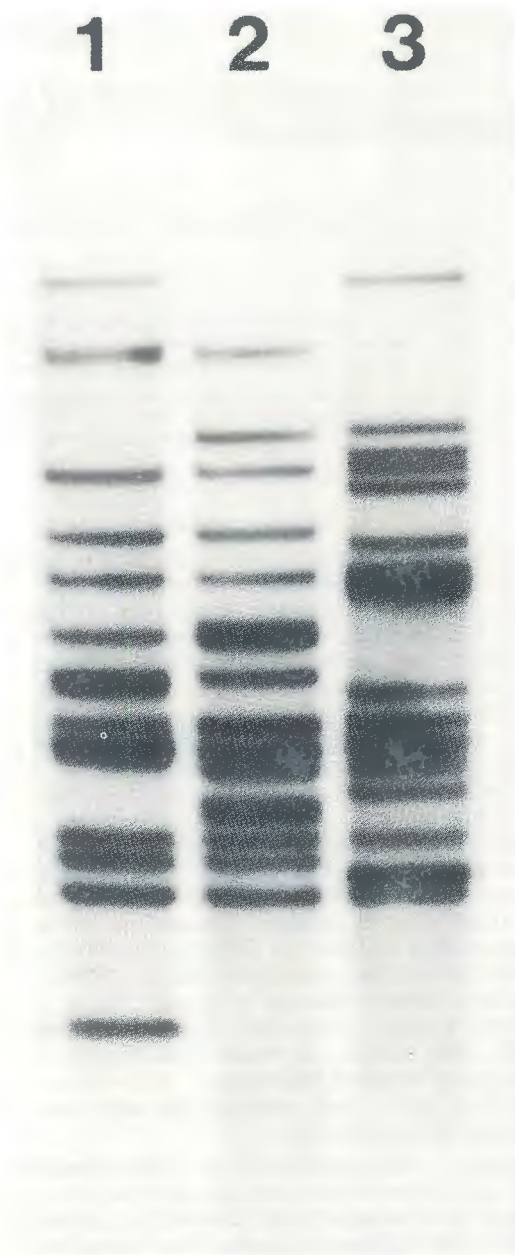


FIG. 2 Hybridization patterns of DNA from *M. paratuberculosis* strains isolated from: 1. cattle, 2. South African sheep, 3. New Zealand sheep

primary culture. In contrast, the other major group consists of strains which have only been recovered from sheep and goats (Collins, Gabric & De Lisle, 1990). They are extremely difficult to grow on primary isolation and as a consequence the geographic distribution of this group is poorly defined. The discovery that the South African sheep strains were identical to a strain from Canada suggests that this intermediate group may also have a wide geographic distribution. There was no known link between the Canadian and South African sheep which had this intermediate type of *M. paratuberculosis*. Examination of further strains is required to

determine whether this intermediate type is specifically adapted to sheep.

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