

# IS900 Restriction Fragment Length Polymorphism (RFLP) Analysis of *Mycobacterium avium* subsp. *paratuberculosis* Isolates from Goats and Cattle in Norway

By B. Djønne<sup>1</sup>, I. Pavlik<sup>2</sup>, P. Svastova<sup>2</sup>, M. Bartos<sup>2</sup> and G. Holstad<sup>1</sup>

<sup>1</sup>Department of bacteriology, National Veterinary Institute, Post Box 8156 Dep., N-0033 Oslo, Norway, <sup>2</sup>Veterinary Research Institute, Hudcova 70, Brno 621 32, Czech republic

**Djønne B, Pavlik I, Svastova P, Bartos M and Holstad G: IS900 restriction fragment length polymorphism (RFLP) analysis of *Mycobacterium avium* subsp. *paratuberculosis* isolates from goats and cattle in Norway. Acta vet. scand. 2005, 46, 13-18.** – In Norway, paratuberculosis has been frequently diagnosed in goats, while cattle have been almost free of the infection. This difference in prevalence between goats and cattle has led to speculations about the existence of a *Mycobacterium avium* subsp. *paratuberculosis* (*M. a. paratuberculosis*) isolate that is non-pathogenic for cattle. There is little information available on genotypic variation of *M. a. paratuberculosis* isolated from animals in Norway. In the present study, genotypic information on 51 isolates from goats and four isolates from cattle in Norway was obtained by use of IS900 restriction fragment length polymorphism (RFLP) analysis. All isolates from cattle and 84% of the isolates from goats had the same RFLP pattern (B-C1). Five RFLP patterns not previously detected were found. No genotypic variation that could explain a difference in host origin was found between the isolates from cattle and the majority of the Norwegian goat isolates. This lack of difference indicates that the most common *M. a. paratuberculosis* isolates in Norway may infect both cattle and goats.

*Mycobacterium avium* subsp. *paratuberculosis*; Strain characterisation; RFLP fingerprint; Goat; Cattle.

## Introduction

Paratuberculosis is a chronic intestinal inflammation in ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (*M. a. paratuberculosis*). The infection is widely distributed in domestic cattle, sheep and goats, and the prevalence varies in different parts of the world (Kennedy & Benedictus 2001).

In Norway, paratuberculosis has been quite common in goats, whereas cattle have been almost free of the infection. From 1966 to 2000, *M. a. paratuberculosis* was isolated in 898 goats from 186 different herds. During the same period, *M. a. paratuberculosis* was iso-

lated only in 20 cattle on 12 different farms (Djønne et al. 2001). The different prevalence of the infection in goats and cattle has led to speculations about the existence of *M. a. paratuberculosis* strains that are non-pathogenic for cattle. Saxegaard (1990) carried out an experimental infection where *M. a. paratuberculosis* isolated from Norwegian goats was administered to cattle. Based on the results of this trial, it was concluded that paratuberculosis in goats in Norway is caused by an apparently specific goat-pathogenic strain of the bacterium.

Variation in virulence between different iso-

lates of a bacterial species can be caused by genetic variation that is detected by phenotypic or genotypic characterisation. *M. a. paratuberculosis* strains from Norwegian goats do not differ phenotypically from strains isolated from cattle in Norway or other parts of the world (Gunnarson & Fodstad 1979). There is little information available on the genotypic variation of *M. a. paratuberculosis* isolated from animals in Norway. Collins et al. (1990) performed genotypic examinations of three *M. a. paratuberculosis* isolates originating from Norwegian goats. Two of these showed marked differences from the 48 other strains from sheep, goat and cattle examined, in that they lacked a repetitive *M. a. paratuberculosis* sequence and also showed a very different restriction fragment pattern compared with the other strains (Collins et al. 1990). Thus, the authors suggested that these Norwegian strains might be uniquely adapted to goats. In other studies, however, only minor genotypic differences between Norwegian goat isolates and strains isolated from cattle in other parts of Europe have been found (Thoresen & Olsaker 1994, Pavlik et al. 1999). Molecular typing has shown that, in comparison with other pathogens, there is relatively little genetic variability in *M. a. paratuberculosis* (Stevenson et al. 2002). Therefore, the potential of many different methods, such as IS900 restriction fragment length polymorphism (RFLP) (Whipple et al. 1990, Collins et al. 1990, Pavlik et al. 1995), pulsed-field gel electrophoresis (PFGE) (Feizabadi et al. 1997, Stevenson et al. 2002), random amplified polymorphic DNA patterns (Scheibl & Gerlach 1997, Pillai et al. 2001), and multiplex PCR typing (Bull et al. 2000) have been investigated. RFLP has been found to be one of the best methods to differentiate between *M. a. paratuberculosis* isolates, and many different RFLP patterns have been found (Whipple et al. 1990, Collins et al. 1990, Pavlik et al. 1995, Moreira

et al. 1999, Pavlik et al. 2000, Cousins et al. 2000, Whittington et al. 2000). In 1999, Pavlik et al. (1999) standardised the RFLP typing and nomenclature of the RFLP types, enabling a comparison of isolates from different parts of the world.

The aim of the present study was to investigate the genotypic variation among *M. a. paratuberculosis* isolates from goats and cattle in Norway, by use of IS900 RFLP analysis.

## Materials and methods

### *M. a. paratuberculosis* strains

Fifty-one *M. a. paratuberculosis* strains from goats and four from cattle were examined; they originated from 51 goat and four cattle herds, and were collected during the period 1983-2000. The goatherds were distributed in Western Norway. Three cattle herds located in Eastern Norway had imported animals from Denmark and Finland in 1993, and the fourth cattle herd, located in Western Norway, had previously had goats with paratuberculosis. From most of these herds, *M. a. paratuberculosis* was isolated more than once, but in this study, only the last detected isolate from each herd was included. From the combined cattle and goatherd, one isolate from cattle and one isolate from goat were included. The strains were isolated from either clinically ill animals or animals in the subclinical stages of paratuberculosis.

### *M. a. paratuberculosis* isolation and identification

The *M. a. paratuberculosis* strains examined were either fresh or low passage number isolates. They had primarily been isolated after cultivation on selective and non-selective Dubos medium with mycobactin (2 µg/ml) and pyruvate (4 mg/ml) as described by Saxegaard (1985). At the time of isolation, the isolates were identified by colony morphology, degree of acid-fast staining with the Ziehl-Neelsen

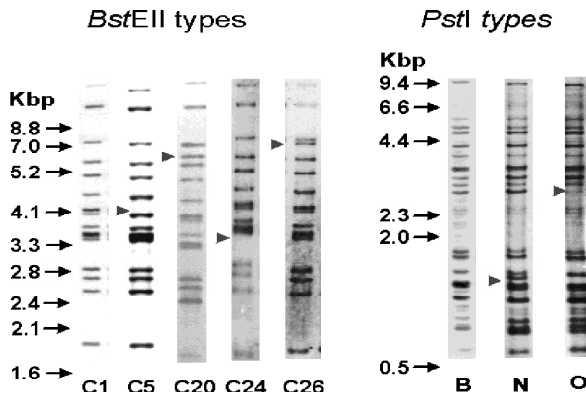


Figure 1. *IS900* restriction fragment length polymorphism patterns detected with the enzymes *BstEII* and *PstI* in *Mycobacterium avium* subsp. *paratuberculosis* isolates from Norwegian cattle and goats.

method and mycobactin dependency. All isolates were nonpigmented and were stored as glycerol stocks at  $-70^{\circ}\text{C}$ . Before further examination, the isolates were confirmed as *M. a. paratuberculosis* by detection of the insertion segment *IS900* by PCR (Sigurðardóttir et al. 1999).

*IS900* restriction fragment length polymorphism analysis (RFLP)

RFLP analysis was performed as described by Pavlik et al. (1999). Briefly, DNA was extracted from the isolates with lysozyme, sodium dodecyl sulfate and proteinase K, purified by chloroform isoamylalcohol extraction and precipitated with isopropylalcohol. The DNA was digested by restriction endonucleases *PstI* and *BstEII* and hybridised with a standard PCR generated *IS900* probe. The DNA fingerprints were analysed and the types were designated as described by Pavlik et al. (1999). The fingerprints were scanned by a CCD camera (UltraLum KS4000, USA), and analysed by Gel Compare software (Applied Maths, Kortrijk, Belgium).

**Results**

Three different profiles were detected when us-

ing restriction endonuclease *PstI* (B, N and O) whereas five profiles were found with *BstEII* (C1, C5, C20, C24 and C26) (Figure 1). The combination of typing with *PstI* and *BstEII* was able to differentiate seven RFLP types (Table 1). All the cattle isolates and 43 goat isolates were type B-C1. Other identified RFLP types were B-C5 (n=2), B-C24 (n=1), B-C26 (n=1), N-C20 (n=2), O-C5 (n=1) and O-C24 (n=1).

**Discussion**

In the present study, all *M. a. paratuberculosis* isolates from cattle and 84% of the isolates from goats in Norway were of the B-C1 RFLP

Table 1. *IS900* restriction fragment length polymorphism patterns detected in Norwegian isolates of *Mycobacterium avium* subsp. *paratuberculosis*.

RFLP pattern <i>PstI BstEII</i>	No. of strains	Isolated from
B C1	4	Cattle
B C1	43	Goat
B C5	2	Goat
B C24	1	Goat
B C26	1	Goat
N C20	2	Goat
O C5	1	Goat
O C24	1	Goat

pattern. Four cattle isolates are very few, but B-C1 is also the most common RFLP pattern detected in cattle in Europe and the United States (Whipple et al. 1990, Pavlik et al. 2000). The B-C1 type was distributed throughout the area where paratuberculosis in goats is common. Five RFLP types found in the present study have not yet been described; these types were O-C24, O-C5, B-C26, N-C20 and B-C24. However, only minor differences in the RFLP patterns were found for these types, and the difference was usually the absence or gain of one band. Except for B-C5 and N-C20, only one isolate of each RFLP type was found. The two N-C20 isolates were from the same district, while the two B-C5 isolates were from two different counties.

RFLP analysis with other restriction enzymes might have enabled a better differentiation of the Norwegian isolates. Cousins et al. (2000) examined Australian *M. a. paratuberculosis* isolates with four different restriction endonucleases, and some additional information was gathered by using the restriction endonucleases *Bam*HI and *Pvu*II in combination with *Bst*EII and *Pst*I.

Thoresen & Olsaker (1994) used RFLP with restriction endonuclease *Pvu*II to analyse 16 isolates from Norwegian goats and five isolates from Danish cattle. All but one goat isolate had the same RFLP pattern. These results are in accordance with the results reported in the present investigation, where the majority of the isolates were identical to the most common isolates from European cattle, although some variation between the different isolates was found. Collins et al. (1990) examined two Norwegian isolates that differed in many aspects from the majority of other *M. a. paratuberculosis* isolates. These strains were so different that it was concluded they might belong to another species.

Other typing methods might have enabled a bet-

ter differentiation of the isolates. Pulsed-field gel electrophoresis (Feizabadi et al. 1997, Stevenson et al. 2002) has been used to differentiate between isolates of *M. a. paratuberculosis*. Stevenson et al. (2002) found that multiplex PFGE gave additional information to RFLP, and they concluded that combining both techniques might improve the discrimination of *M. a. paratuberculosis* isolates.

Our investigation did not detect any genotypic variation between the isolates from cattle and the majority of the Norwegian goat isolates. This lack of genetic variation may indicate that the most common strain of *M. a. paratuberculosis* in Norway is able to infect both cattle and goats. This finding is in accordance with observations from other countries, where *M. a. paratuberculosis* isolates from one animal species are known to infect others. In the Netherlands, sheep grazing on the same pastures as cattle infected by *M. a. paratuberculosis* were also found to be infected (Muskens et al. 2001). In Iceland and the Czech Republic, infection from sheep to cattle has been reported (Pavlik et al. 1995, Fridriksdottir et al. 2000, Whittington et al. 2001), and in the Czech Republic transmission of *M. a. paratuberculosis* from pastured cattle to free living wild ruminants has been documented (Pavlik et al. 2000). Our observations do not exclude that these *M. a. paratuberculosis* isolates have different pathogenicity for cattle and goats, as RFLP might not detect the genetic background for this difference. However, there are factors other than strain specificities that should be considered when evaluating the pathogenicity of *M. a. paratuberculosis* for cattle and goats. These factors include management conditions and breed resistance. The management conditions are quite different for cattle and goats in Norway; small cattle units, early separation of calves from their mothers, and a low average age of the cows (Holstad et al. 2005). All of

these management factors have been shown to reduce the spread of infection in a herd (Johnson-Ifearulundu & Kaneene 1998, Obasanjo et al. 1997, Rossiter & Burhans 1996). The goat kids, however, are often born in pens where several goats are housed, and they might suckle several dams. Therefore, the risk of being exposed to faecal material from a bacterial shedder are higher in goats than in cattle.

Paratuberculosis was considered to be a clinical problem in the Norwegian cattle population during the first part of the 20th century. At that time, different local cattle breeds made up the cattle population in Norway. After 1970, the majority of the population was drawn from the Norwegian red cattle breed, which is a hybrid of many different breeds, and speculations that the Norwegian red cattle breed is more resistant to clinical infection with *M. a. paratuberculosis* than the local breeds have been put forward (Holstad et al. 2005).

#### Acknowledgements

We thank Dr. Finn Saxegaard, Sigrun Frensvold Nilsen and Nina Fundingsrud for isolating *M. a. paratuberculosis*.

#### References

- Bull TJ, Hermon-Taylor J, Pavlik I, El-Zaatari F, Tizard M: Characterization of IS900 loci in *Mycobacterium avium* subsp. *paratuberculosis* and development of multiplex PCR typing. *Microbiology*. 2000, 146, 2185-97.
- Collins DM, Gabric DM, de Lisle GW: Identification of two groups of *Mycobacterium paratuberculosis* strains by restriction endonuclease analysis and DNA hybridization. *J. Clin. Microbiol.* 1990, 28, 1591-96.
- Cousins DV, Williams SN, Hope A, Eamens GJ: DNA fingerprinting of Australian isolates of *Mycobacterium avium* subsp. *paratuberculosis* using IS900 RFLP. *Aust. Vet. J.* 2000, 78, 184-90.
- Djønne B, Fredriksen B, Nyberg O, Sigurðardóttir ÓM, Tharaldsen J: National bovine paratuberculosis program in Norway. *Bull. Int. Dairy. Fed.* 2001, 364, 75-80.
- Feizabadi MM, Robertson ID, Hope A, Cousins DV, Hampson DJ: Differentiation of Australian isolates of *Mycobacterium paratuberculosis* using pulsed-field gel electrophoresis. *Aust. Vet. J.* 1997, 75, 887-89.
- Fridriksdóttir V, Gunnarsson E, Sigurdarson S, Gudmundsdóttir KB: Paratuberculosis in Iceland: epidemiology and control measures, past and present. *Vet. Microbiol.* 2000, 20, 263-67.
- Gunnarson E, Fodstad FH: Cultural and biochemical characteristics of *Mycobacterium paratuberculosis* isolated from goats in Norway. *Acta Vet. Scand.* 1979, 20, 122-34.
- Holstad G, Sigurðardóttir Ó, Storset A, Tharaldsen J, Nyberg O, Schönheit J, Djønne B: Description of the infection status in a Norwegian cattle herd naturally infected by *Mycobacterium avium* subsp. *paratuberculosis*. *Acta Vet. Scand.* 2005, 46, 00-00.
- Johnson-Ifearulundu YJ, Kaneene JB: Management-related risk factors for *M. paratuberculosis* infection in Michigan, USA, dairy herds. *Prev. Vet. Med.* 1998, 37, 41-54.
- Kennedy DJ, Benedictus G: Control of *Mycobacterium avium* subsp. *paratuberculosis* infection in agricultural species. *Rev. Sci. Tech.* 2001, 20, 151-79. Review.
- Manning EJB, Collins MT: *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. *Rev. Sci. Tech. Off. Int. Epiz.* 2001, 20, 133-50.
- Moreira AR, Paolicchi F, Morsella C, Zumarraga M, Cataldi A, Fabiana B, Alicia A, Piet O, van Soolingen D, Isabel RM: Distribution of IS900 restriction fragment length polymorphism types among animal *Mycobacterium avium* subsp. *paratuberculosis* isolates from Argentina and Europe. *Vet. Microbiol.* 1999, 70, 251-59.
- Muskens J, Bakker D, de Boer J, van Keulen L: Paratuberculosis in sheep: its possible role in the epidemiology of paratuberculosis in cattle. *Vet. Microbiol.* 2001, 78, 101-9.
- Obasanjo IO, Grohn YT, Mohammed HO: Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State Paratuberculosis Control Program. *Prev. Vet. Med.* 1997, 32, 243-51.
- Pavlik I, Bejckova L, Pavlas M, Rozsypalova Z, Koskova S: Characterization by restriction endonuclease analysis and DNA hybridisation using IS900 of bovine, ovine, caprine and human dependent strains of *Mycobacterium paratuberculosis* isolated in various localities. *Vet. Microbiol.* 1995, 45, 311-18.

- Pavlik I, Horvathova A, Dvorska L, Bartl J, Svastova P, du Maine R, Rychlik I: Standardisation of restriction fragment length polymorphism analysis for *Mycobacterium avium* subspecies *paratuberculosis*. J. Microbiol. Methods. 1999, 38, 155-67.
- Pavlik I, Bartl J, Dvorska L, Svastova P, du Maine R, Machackova M, Yayo Ayele W, Horvathova A: Epidemiology of paratuberculosis in wild ruminants studied by restriction fragment length polymorphism in the Czech Republic during the period 1995-1998. Vet. Microbiol. 2000, 77, 231-51.
- Pillai SR, Jayarao BM, Gummo JD, Hue EC, Tiwari D, Stabel JR, Whitlock RH: Identification and sub-typing of *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium avium* subsp. *avium* by randomly amplified polymorphic DNA. Vet. Microbiol. 2001, 79, 275-84.
- Rossiter CA, Burhans WS: Farm-specific approach to paratuberculosis (Johne's disease) control. Vet. Clin. North Am. Food Anim. Pract. 1996, 12, 383-415.
- Saxegaard F: Isolation of *Mycobacterium paratuberculosis* from intestinal mucosa and mesenteric lymph nodes of goats by use of selective Dubos medium. J. Clin. Microbiol. 1985, 22, 312-13.
- Saxegaard F: Experimental infection of calves with an apparently specific goat-pathogenic strain of *Mycobacterium paratuberculosis*. J. Comp. Pathol. 1990, 102, 149-56.
- Scheibl P, Gerlach GF: Differentiation of *Mycobacterium paratuberculosis* isolates by rDNA-spacer analysis and random amplified polymorphic DNA patterns. Vet. Microbiol. 1997, 57, 151-58.
- Sigurðardóttir OG, Press CMCL, Saxegaard F, Evensen O: Bacterial isolation, immunological response, and histopathological lesions during the early subclinical phase of experimental infection of goat kids with *Mycobacterium avium* subsp. *paratuberculosis*. Vet. Pathol. 1999, 36, 542-50.
- Stevenson K, Hughes VM, de Juan L, Inglis NF, Wright F, Sharp JM: Molecular characterization of pigmented and nonpigmented isolates of *Mycobacterium avium* subsp. *paratuberculosis*. J. Clin. Microbiol. 2002, 40, 1798-1804.
- Thoresen OF, Olsaker I: Distribution and hybridization patterns of the insertion element IS900 in clinical isolates of *Mycobacterium paratuberculosis*. Vet. Microbiol. 1994, 40, 293-303.
- Whipple D, Kapke P, Vary C: Identification of restriction fragment length polymorphisms in DNA from *Mycobacterium paratuberculosis*. J. Clin. Microbiol. 1990, 28, 2561-64.
- Whittington RJ, Hope AF, Marshall DJ, Taragel CA, Marsh I: Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis*: IS900 restriction fragment length polymorphism and IS1311 polymorphism analyses of isolates from animals and a human in Australia. J. Clin. Microbiol. 2000, 38, 3240-48.
- Whittington RJ, Taragel CA, Ottaway S, Marsh I, Seaman J, Fridriksdóttir V: Molecular epidemiological confirmation and circumstances of occurrence of sheep (S) strains of *Mycobacterium avium* subsp. *paratuberculosis* in cases of paratuberculosis in cattle in Australia and sheep and cattle in Iceland. Vet. Microbiol. 2001, 79, 311-22.

### Sammendrag

*Genotypisk variasjon mellom Mycobacterium avium subsp. paratuberculosis isolat fra geit og storfe i Norge.*

Paratuberkulose er relativt vanlig på geit i Norge, mens storfe derimot har vært nærmest fri for infeksjonen. Denne ulikheten i forekomst av paratuberkulose har ført til spekulasjoner om det finnes en strikt geitepatogen stamme av *Mycobacterium avium* subsp. *paratuberculosis* i Norge. Det er lite informasjon tilgjengelig om genotypisk variasjon mellom stammer av *M. a. paratuberculosis* isolert fra dyr i Norge. I dette arbeidet benyttes RFLP metoden til genotypisk karakterisering av 51 isolat fra Norske geiter og 4 isolat fra Norske storfe. Alle isolat fra storfe og 84% av isolatene fra geit hadde samme RFLP mønster (B-C1). Fem isolat hadde RFLP mønster som ikke tidligere er blitt funnet. Det ble ikke funnet genotypisk variasjon mellom isolatene fra storfe og majoriteten av isolatene fra geit, dette kan tyde på at den mest vanlige *M. a. paratuberculosis* stammen i Norge kan infisere både geit og storfe.

(Received December 23, 2003; accepted February 3, 2005).

Reprints may be obtained from: B. Dønne, National Veterinary Institute, PO Box 8156 Dep., N-0033 OSLO, Norway. E-mail: berit.donne@vetinst.no, tel: +47 23 21 63 23, fax: +47 23 21 63 01.