

# PCR-restriction endonuclease analysis for strain typing of *Mycobacterium avium* subsp. *paratuberculosis* based on polymorphisms in IS 1311

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## Introduction

*Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) is the causative agent of The Johne's disease, a chronic and incurable disease affecting many ruminants. Furthermore, *M. paratuberculosis* has been suggested as an etiological agent of Crohn's disease, a chronic infection of the human intestines but this is controversial. Mobile genetic elements known as insertion sequences (IS) are popular targets for polymerase chain reaction (PCR) tests for many mycobacterial disease. In this study we used the IS1311 PCR/REA for typing of Map isolates.

## Materials and Methods

A total of 68 feces samples were randomly obtained from Keneh Bist Dairy Farm (Mashhad), supposed to have high number of infected animals, according to previous records. Feces were collected from the rectums of animals with high attention on cross contamination. Genomic DNA was extracted from 100 µl of blood according to Boom R. et al method, which was modified by Shaikhaev. For amplification of *M. paratuberculosis* DNA from fecal and milk extracts we used IS1311 specific primers: M56 (gcg tga ggc tct gtg gtg aa) and M94 (cag cga tcg tcg aca gtg tg). An aliquot (10 µl) of the DNA samples was added to 10 µl of PCR mixture. Amplification conditions for IS900 were: 3 min at 94 °C, 40 cycles of 35 s at 94 °C, 25 s at 62 °C, 1 min at 72 °C and a final 5 min extension at 72 °C. PCR products were analyzed through the electrophoresis of 5 µl of each sample on 2% (W/V) agarose gels. The positive samples with 268 bp amplified band were considered for REA (Restriction Endonuclease Analysis). Restriction endonuclease analysis reactions were prepared by adding 10 µl of PCR product, 2 U of the *Hinf*I restriction endonuclease enzyme (Sibenzyme, Russia), 2 µl of 10X buffer (supplied with enzyme) and made up to 20 µl with sterile purified water. Restriction digests were incubated for 2 h at 37 °C and were assessed by electrophoresis in 4% agarose gel containing ethidium bromide.

## Results and Discussion

PCR/REA strategy based on amplifying a 268 bp fragment of IS1311 and digestion by *Hinf*I was developed. Results showed that all of positive results were assigned to cattle strain (C). This simple and rapid test can be used on a range of diagnostic samples for the confirmation of Johne's disease and will be of benefit in control and eradication programs for this disease. Although we could not detect any S strain in this farm but its detection is possible if sample size increased and sampling is done from different farms.

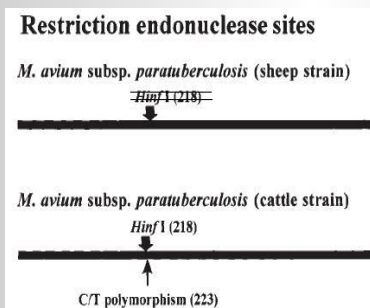


Fig 2. IS1311 PCR REA by *Hinf*I restriction endonuclease.

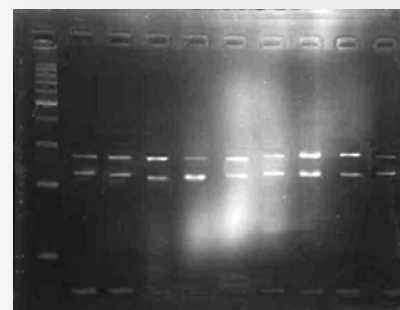


Fig 1. IS1311 PCR product digested with *Hinf*I restriction endonuclease.