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Restriction Endonuclease Analysis of Local *Brucella* Isolates

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Abstract: Six *Brucella* isolates isolated from local sheep and cattle were examined for the cleavage patterns of their genome by *Hin*dIII restriction endonuclease. The DNA fragment patterns obtained from each isolates were compared with reference strains of *Brucella melitensis* 16M and *Brucella abortus* strain 99. Eight fragments bands were resolved above 10, 000 base pairs DNA molecular weight markers. It was observed that the DNA fragment patterns of all isolates were similar.

Key words: *Brucella*, restriction endonuclease analysis, *Hin*dIII

Introduction

Brucellosis is a bacterial disease caused by pathogenic members of the genus *Brucella*. This disease is an infectious disease and a zoonosis disease. The genus *Brucella* is classified into six species; *Brucella abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*. The classification is based on the growth and biochemical characteristics. The *Brucella* genus appears to be relatively homogenous when examined by either DNA-DNA homology (Hoyer and McCullough, 1968a, b) or polyacrylamide gel electrophoresis of proteins (Morris, 1972). Biochemical tests have been used to distinguished different biotypes of *Brucella abortus*, *Brucella suis* and *Brucella melitensis*. In recent years, the application of new technologies has improved the knowledge on the organisms. Restriction endonuclease DNA analysis has the potential to detect very small differences between closely-related strains (O'hara *et al.*, 1985). Restriction endonucleases cleave the DNA at specific nucleotide sequences and produce a set of DNA fragments which, when separated by electrophoresis provide a characteristic band pattern or fingerprint of the respective genome (Kim and Nagaraja, 1990 and Diallo *et al.*, 1995). In this present study, restriction endonuclease analysis (REA) by *Hin*dIII was used to determine any strain variation of *Brucella* isolates.

Materials and Methods

Bacterial isolates: Five *Brucella* isolates from sheep and one *Brucella* isolate from cattle were used in this study. The isolates were isolated from clinical cases. Reference strains of *Brucella melitensis* 16M and *Brucella abortus* strain 99 were obtained from the American Type Culture Collection (ATCC). All isolates were grown and maintained by weekly subculture on *Brucella* agar (Pronadisa, Spain) at 37°C. The isolates were identified by standard methods as described by Alton *et al.* (1975).

Restriction endonuclease analysis: Genomic DNA extraction. The cultures were initially grown on *Brucella* agar plates and incubated at 37°C for 96 hours. The bacterial cells were harvested using 1 ml of phosphate buffer saline (PBS) pH 7.2 and transferred into a 1.5 ml sterile microcentrifuge tube. The extraction and purification of *Brucella* DNA was carried out using Wizard® Genomic DNA purification kit (Promega, USA). The final concentration and quality of extracted DNA were measured at 260 nm and 280 nm using a spectrophotometer.

Restriction enzyme digestion. A volume of 10 μl purified DNA samples (5 μg/μl) were digested with 5 U of restriction endonuclease *Hin*dIII (MBI Fermentas, USA) for three hours at 37°C.

Agarose gel electrophoresis. Two microliters of loading dye (6X, MBI Fermentas, USA) were added into 10 μl of digested DNA samples. The samples were then loaded into 0.7% agarose gel. The gel was electrophoresed in Tris-acetate EDTA buffer (TAE; 40 mM Tris base, 20 mM acetic acid, 1 mM EDTA pH 8.5) at 30 volts for 18 hours. A 1kb DNA ladder (MBI Fermentas, USA) was included in the gel as a standard molecular size marker. After electrophoresis, the gel was stained with ethidium bromide (0.25 μg/ml) solution for 30 minutes and visualized with Gel Doc programme (Bio-Rad, USA).

Results

Brucella colonies are found to be round, 1-2 mm in diameter, with smooth margins, translucent and a pale honey colour when plates are viewed towards a light source. Modified Ziehl-Neelsen staining method (Stamp *et al.*, 1950)

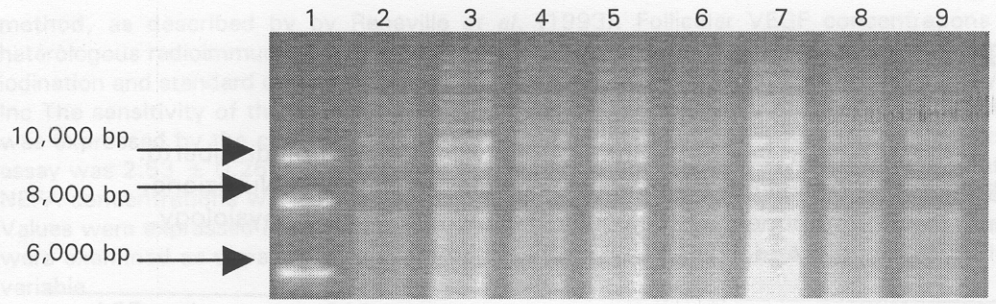

Fig. 1: DNA fragment patterns of local Brucella isolates digested by HindIII. Lanes:1, DNA ladder; 2, Brucella melitensis 16M; 3, Brucella abortus strain 99; 4, Brucella isolate from cattle; 5-9, Brucella isolates from sheep

was carried out to stain the organism. *Brucella* cells appeared as small and Gram-negative coccobacilli. Motility test showed that the organisms were non-motile. It was catalase positive and showed variable oxidase reactions in oxidase test. They produce urease, reduce nitrates, do not react in MR and VP tests and produce variable changes in litmus milk. The organisms showed negative results on indole and gelatin liquefaction tests.

There were no differences in the DNA fingerprinting of the 6 local *Brucella* isolates including the two *Brucella* reference strains when digested with *Hind*III restriction endonuclease(Fig. 1).

Discussion

Restriction endonuclease analysis of genomic DNA is now an established technique for the study of molecular epidemiology of bacterial infection. The use of direct nucleic acid has been particularly valuable for strain differentiation and allows the monitoring of distribution of strain. The present study utilized restriction endonuclease *Hind*III in REA on local *Brucella* isolates. *Hind*III is one of the most commonly used frequent-cutting restriction endonuclease and recognizes 6 base pairs sites. As previously studied by O'hara *et al.* (1985), the commonly used restriction endonuclease such as *Eco*RI and *Hind*III demonstrate only minor differences between *Brucella* strains. This study showed that these isolates are from the genus *Brucella* due to the similarity and the lack of detectable differences of DNA fragment patterns when compared to reference strains *Brucella melitensis* 16M and *Brucella abortus* strain 99. It was showed that all isolates were very closely related.

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