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Characterization of leptospiral isolates using random amplified polymorphic DNA (RAPD) fingerprinting

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ABSTRACT

Random amplified polymorphic DNA (RAPD) fingerprinting was applied to characterize leptospiral strains. Two primers were used to generate individual RAPD fingerprints. The DNA fingerprints obtained were distinct and reproducible. The fingerprints obtained could be useful for distinguishing and characterizing the strains. Profiles obtained revealed genetic heterogeneity between strains belonging to one serovar. In conclusion, different DNA fingerprints were obtained which would allow characterization of the strains.

Key words: RAPD, leptospira, serovar, characterization

Introduction

Leptospirosis, caused by *Leptospira interrogans*, is a disease of animals and humans which has a worldwide distribution (ZUERNER and BOLIN, 1988). *Leptospira interrogans* is a diverse species and contains several distinct genetic groups (JOHNSON and FAINE, 1984). The identification and differentiation of leptospires are important for epidemiological and public health surveillance, as different serovars exhibit different host specificities and may be associated with a particular clinical form of infection (FAINE, 1982). Rapid identification and characterization of leptospiral isolates would allow epidemiological data to be rapidly gathered and assessed by the institution for appropriate control measures (CORNEY et al., 1993). Identification and characterization of leptospiral serovars have been carried out traditionally using serological techniques (DIKKEN and

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KMETY, 1978; JOHNSON and FAINE, 1984). However, these assays are tedious, time consuming and are not always reproducible (ELLIS et al., 1991). Introduction of random amplified polymorphic DNA (RAPD) for leptospires differentiation led to production of highly diverse DNA banding patterns which may allow discrimination between and within serovars (RALPHA et al.,1993; BROWN and LEVETT, 1997; SUBARNA et al., 2003; VIJAYACHARIT et al., 2004; SUBARNA et al., 2004). This method was found to be a most potentially useful approach for clinical characterization of leptospires because of its simplicity (NATARAJASEENIVASAN et al., 2004**)**. The main objective of this study was to characterize leptospiral isolates based of DNA fingerprinting using random amplified polymorphic DNA (RAPD) PCR.

Materials and methods

Leptospiral strains. Ten field isolates and two reference strains were used in this study (Table 1).

Table 1. Leptospiral field isolates and reference strains used in the study

Serovars	Strain	Source of Isolation
Portland-vere	Ca-12-002	Human
	Ca-12-005	Dog
Copenhageni	Ic-02-003	Pig
	Ic-02-004	Cattle
Hardjo	HB-15B-012	Cattle
	HB-15B-013	Cattle
Grippotyphosa	RM 52	Pig
	Gr-01-002	Cattle
Kennewicki	RM211	Pig
	Po-06-013	Pig
Canicola	Ca-rf	Reference strain
Hardjo	HB-re	Reference strain

Field isolates were isolated from clinical incidents of leptospirosis from different animal species in the USA. The reference strains were obtained from the WHO Leptospira Reference Laboratory, Australia. The field isolates were identified serologically based on microscopic agglutination test. Field isolates and reference strains were cultured in Johnson-Seiter (JS) liquid medium. The cultures were then incubated for up to 10 days at 30 ^{0}C and used directly for DNA extraction.

Preparation of Leptospiral DNA. Genomic DNA was extracted from the leptospiral reference strains and field isolates using the commercial genomic DNA purification kit (Wizard R, Promega, USA). The extraction method of DNA was performed as described by the manufacturer.

RAPD-PCR primers. Two primers (Operon Technologies) were chosen for RAPD analysis for all strains examined. The two single primers used in PCR analysis were primer 1: (OPA 3: 5'- AGTCAGCCAC) and primer 2: (OPA 20: 5'- GTTGCGATCC).

RAPD-PCR. APD fingerprinting was performed according to RAMADASS et al. (1997), with some modifications. The reaction was carried out in a final volume of 50 µl containing 3 mM of $MgCl_2$, 200 µM of each dNTP, one unit of Tag DNA-polymerase, 5 µ 10x reaction buffer, (Amersco, USA), and 20-30 ng of genomic DNA. Concentration of each primer was 1 µM (Amersco, USA). Reaction mixtures were covered with 30 µl of mineral oil and placed in a thermocycler (Thermojet) for amplification. Temperature program consisted of 1 cycle for 3 m at 94 °C followed by 39 cycles consisting of 1 m at 94 °C, 1 m at 35 °C, and 75 sec at 72 °C. This was followed by a final extension of 10 m at 72 °C.

Gel electrophoresis of amplification products. PCR amplification products were analyzed by running 10 µl of the reaction product on 2% agarose gel and detected by staining with ethidum bromide. The DNA ladder (GIBCO BRL, USA) was used as DNA size markers.

Results

The two primers were successfully amplified polymorphic DNA fragments from most of the strains tested. Fingerprints obtained with each primer were distinctive and reproducible. Using primer OPA 3, seven different fingerprinting profiles could be distinguished from the 10 field isolates tested (Figs 1a and 1b). The two isolates from serovar hardjo produced identical fingerprints, which was different from the hardjo reference strain. The two isolates from serovar kennewicki produced identical fingerprints. The two isolates from serovar portland-vere produced the same fingerprinting profile, which was different from the canicola reference strain but with some shared bands. Two isolates each from serovars copenhageni and grippotyphosa produced different fingerprints with this primer. Random amplified polymorphic DNA (RAPD) PCR with primer OPA 20 generated 8 fingerprinting profiles (Fig. 2). The two serovar hardjo isolates produced identical fingerprints, which were different from the reference strain. Again, like primer OPA 3, the two isolates of serovar kennewicki produced identical fingerprinting profiles. The two isolates of serovar portland-vere which produced identical DNA profiles with primer OPA 3 produced different profiles with primer OPA 20. The two isolates each from serovar copenhageni and serovar grippotyphosa produced different fingerprints with this primer.

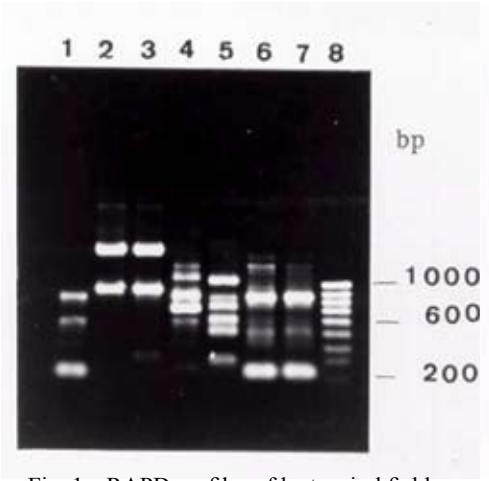

Fig. 1a. RAPD profiles of leptospiral field isolates obtained with Primer OPA 3 and electrophoresed on 2% agarose gel. Lanes: 1. Hardjo reference strain; 2. HB-15B-012; 3. HB-15B-013; 4. RM 52; 5. Gr-01-002; 6. RM 211; 7. Po-06-013, 8. Molecular size markers.

Fig. 1b. RAPD profiles of leptospiral field isolates obtained with Primer OPA 3 and electrophoresed on 2% agarose gel. Lanes: 1. Canicola reference strain; 2. Ca-12-002; 3. Ca-12-005; 4. Ic-02-003; 5. Ic-02-004; 6. Molecular size markers.

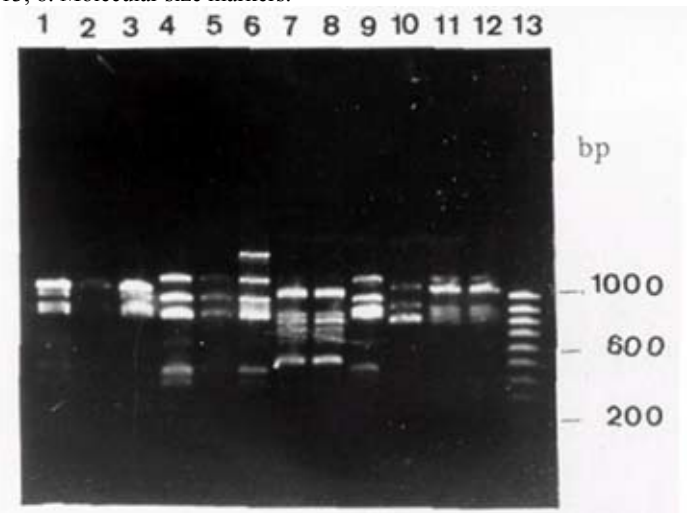

Fig. 2. RAPD Profiles of leptospiral field isolates obtained with Primer OPA 20 and electrophoresed on 2% agarose gel. Lanes: 1. Canicola reference strain; 2. Ca-12-002; 3. Ca-12-005; 4. Ic-02-003; 5. Ic-02-004; 6. hardjo reference strain; 7. HB-15B-012; 8. HB-15B-013; 9. RM 52; 10. Gr-01-002; 11. RM 211; 12. Po-06-013, 13. Molecular size markers.

Discussion

In the present study, the RAPD assay was applied to characterize leptospiral field isolates isolated from different animal species based on DNA fingerprints. RAPD-PCR generated reproducible fragment profiles on agarose gel which were characteristic of each leptospiral strain. These different DNA fingerprints obtained by the field strains indicated genetic heterogeneity between strains belonging to one serovar. The second primer increased the number of fingerprints, indicating that fingerprinting may be increased even further by using additional primers. The two isolates of serovar hardjo produced identical banding patterns with each primer and also with the two isolates of serovar kennewicki. The same fingerprinting profiles suggested that the two isolates of serovar hardjo were identical, as were the two isolates of serovar kennewicki. The two isolates of serovar portland-vere produced identical fingerprints with primer OPA 3, and different patterns with primer OPA 20, indicating that the isolates of the same serovar isolated from different species may have different DNA fingerprinting profiles with a different primer. CORNEY et al. (1997) found that type strains of serovars ballum and arborea had identical profiles with one primer (L10) but differed with another primer (US). On the other hand, NATARAJASEENIVASAN et al. (2005) reported that strains belonging to serovar ratnapura that caused hepato-renal involvement in patients in South India were genetically dissimilar to strains of the same serovar isolated from patients in the Andamans who had pulmonary complications. The two isolates of serovar hardjo were isolated from cattle and, similarly, the two isolates of serovar kennewicki were isolated from pigs. The two isolates of serovar hardjo had DNA fingerprinting profiles different from the reference strain profile. This was attributed to the fact that that the two field isolates belonged to genotype hardjobovis, while the reference strain belonged to hardjoprajitno. The reference strain of serovar canicola had a different fingerprinting profile from the two isolates of serovar portland-vere, although the two serovars belong to serogroup Canicola, but still there were common shared bands between the field isolates and Canicola reference strain. It would appear that the DNA profile of different leptospiral field isolates would be useful for differentiation of strains belonging to one or different serovars.

References

BROWN, P. D., P. N. LEVETT (1997): Differentiation of leptospira species and serovars by PCR, restriction endonuclease analysis, arbitrary primed PCR and low stringency PCR. J. Med. Microbil. 46,173-181.

CORNEY, B. G., J. COLLEY, G. C. GRAHAM (1997): Simplified analysis of pathogenic leptospiral serovars by random amplified polymorphic DNA fingerprinting. J. Med Microbiol. 46, 927-932.

CORNEY, B. G., J. COLLEY, S. P. DJORDJEVIC, R. WHITTINGTON, G. C. GRAHAM (1993): Rapid identification of some Leptospira isolates from cattle by random amplified polymorphic DNA fingerprinting. J. Clin. Microbiol. 31, 2927-2932.

DIKKEN, H., E. KMETY (1978): Serological typing methods of leptospirosis. Meth. Microbiol. 11, 259-307.

ELLIS, W. A., J. MONTGOMERY, A. B. THIERMANN (1991): Restriction endonuclease analysis as a taxonomic tool in the study of pig isolates belonging to the australis serogroup of *Leptospira interrogans*. J. Clin. Microbiol. 29, 957-961.

FAINE, S. (1982): Guidelines for the control of leptospirosis. WHO Offset Publication No. 67, World Health Organization, Geneva.

JOHNSON, R., S. FAINE (1984): Family II. *Leptospiraceae*. In: Bergey's Manual of Systematic Bacteriology, (Krieg, N. R., J. G. Holt, Eds.). The Williams and Wikins Co., Baltimore.

NATARAJASEENIVASAN, K., P. VIJAYACHARIT, S. SHARMA, S. ROY, A. P. SUGUNAN, D. BISWAS, S. C. SEHGAL (2005): Phylogenetic relatedness among leptospiral strains belonging to same serovar recovered from patients with different clinical syndromes. Infect. Gene. Evol. 5, 185-191.

NATARAJASEENIVASAN, K., N. PRABHU, K. SELVANAYAKI, S. S. RAJA, S. RATNAM (2004): Human leptospirosis in Erode, South India: serology, isolation, and characterization of the isolates by randomly amplified polymorphic DNA (RAPD) fingerprinting. Jpn. J. Infec. Dis. 57,193-197.

RALPHA, M., M. MCCLELLAND, J. WELSH, G. BARANTON, P. PEROLAT (1993): Leptospira species categorized by arbitrary primed polymerase chain reaction (PCR) and by mapped restriction polymorphism in PCR-amplified rDNA genes. J. Bacteriol. 175, 973-981.

RAMADASS, P., S. MEERARANI, M. D. VENKATESHA, A. SENTHILKUMAR, K. NACHIMUTHU (1997): Characterization of leptospiral serovars by randomly amplified polymorphic DNA fingerprinting. J. Sys. Bacteriol. 47, 575-576.

SUBARNA, R., D. BISWAS, P. VIJAYACHARI, A. P. SUGUNAN, S. C. SEHGAL (2003): Antigenic and genetic relatedness of Leptospira strains isolated from the Andaman Islands in 1929 and 2001. J. Med. Microbiol. 52, 909-911.

SUBARNA, R., D. BISWAS, P. VIJAYACHARI, A. P. SUGUNAN, S. C. SEHGAL (2004): A 22-mer primer enhances discriminatory power of AP-PCR fingerprinting technique in characterization of leptospires. Tropic. Medic. Intern. Helh. 9, 1203-1209 .

VIJAYACHARIT, P., A. R HARTSKEERL, S. SHARMA, K. NATARAJASEENIVASAN, S. ROY, W. J. TERPSTRA, S. C. SEHGAL (2004): A unique strain of Leptospira isolated from a patient with pulmonary hemorrhages in the Andaman Islands: a proposal of serovar portblairi of serogroup sehgali. Epidemiol. Infect. 32, 663-673.

ZUERNER, R. L., C. A. BOLIN (1988): Repetitive sequence element cloned from *Leptospira interrogans* serovar hardjo type hardjobovis provide a sensitive diagnostic probe for bovine leptospirosis. J. Gen. Microbiol. 26, 2495-2500.

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SAŽETAK

Postupak otiska nasumce umnožene polimorfne DNA primijenjen je za karakterizaciju sojeva leptospira. Dvije početnice rabljene su za proizvodnju zasebnih otisaka umnožene polimorfne DNA. Dobiveni otisci DNA bili su međusobno različiti i reproducibilni te bi se mogli rabiti za razlikovanje i karakterizaciju sojeva. Njihovi profili pokazali su genetsku heterogenost među sojevima koji su pripadali jednom serovaru. Zaključno se može reći da su dobiveni različiti otisci DNA koji omogućuju karakterizaciju sojeva leptospira.

Ključne riječi: RAPD, leptospire, serovar, karakterizacija