VETERINARSKI ARHIV 73 (6), 315-321, 2003

PCR-based randomly amplified polymorphic DNA (RAPD) fingerprinting for detection of genetic diversity among Sudanese isolates of *Haemophilus somnus*

Kamaledin Eltoum,[1] Imadeldin Aradaib[2], and Suliman El-Sanousi[1]*

[1] *Institute for Promotion of Animal Export, University of Khartoum, Khartoum, Sudan*

[2]*Molecular Biology Laboratory, Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Khartoum North, Sudan*

ELTOUM, K., I. ARADAIB, S. EL-SANOUSI: PCR-based randomly amplified polymorphic DNA (RAPD) fingerprinting for detection of genetic diversity among Sudanese isolates of *Haemophilus somnus*. Vet. arhiv 73, 315-321, 2003.

ABSTRACT

Haemophilus somnus is a pathogen frequently associated with infectious thromboembolic meningoencephalitis in cattle. Five strains of *H. somnus* were isolated from clinically infected cattle. In the Sudan the organism is associated with a clinical syndrome characterized by nervous manifestations. The strains were found to be morphologically and serologically different as determined by cultural and biochemical characteristics. In the present study, a polymerase chain reaction (PCR)-based randomly amplified polymorphic DNA (RAPD) method was used to amplify *H. somnus* DNA using arbitrary oligonucleotide primers. The method was able to detect the heterogeneity of amplified DNA from the strains of *H. somnus.* The scientific data presented in this study suggests that RAPD-PCR could be used as a valuable tool in the study of the the molecular epidemiology of *H. somnus* isolated from clinical samples of naturally infected cattle.

Key words: *Haemophilus somnus*, randomly amplified polymorphic DNA (RAPD), PCR, Sudan

Introduction

Haemophilus somnus is a pathogen frequently associated with respiratory and nervous signs. The disease constitutes one of the major veterinary problems in the Sudan (ARADAIB and ABBAS, 1985). The pathogen

* Contact address:
Dr. Suliman El-Sanousi, Institute for Promotion of Animal Export; University of Khartoum, P.O.Box 32, Khartoum North, Sudan, Phone: +249 123 90992; Fax: +249 133 12638.

ISSN 0372-5480
Printed in Croatia

is capable of undergoing structural and antigenic variation in its lipooligosaccharide components after *in vivo* and *in vitro* passage (INZANA et al., 1997). Cultivation and subsequent identification by biochemical characterization and serotyping is routinely used for conventional bacteriological examinations. The isolated organisms can be identified by the major antigens common to all isolates (DERKS et al., 1973; SHIGIDI and HOERLEIN, 1970). Currently, little information is available about the biology, ecology and molecular epidemiology of the organism. DNA polymorphisms as a genetic marker was demonstrated among different *H. somnus* isolates using PCR-based RAPD (APPUHAMY et al., 1997). Recently, a polymerase chain reaction (PCR) was described for specific identification of *H. somnus,* by ANGEN (1998).

In the present investigation, the PCR-based RAPD fingerprinting method, as a genetic marker to detect genetic variations among strains of *H. somnus,* was employed using different pairs of arbitrary primers.

Materials and methods

Strains of bacteria. Five strains of *H. somnus* were isolated from brains of clinically infected cows showing symptoms of nervous manifestations. The isolates were purified by single colony picks three times. The biochemical characterizations of these isolates were based on DERKS et al., 1973.

Bacterial growth and DNA extraction. Haemophilus organisms were grown in Brain Heart Infusion broth (Difco Laboratory, Detroit, MI) and incubated at 37 ^{0}C. Total genomic DNA was extracted from the bacterial isolates. In this study, whole DNA was used without initial purification. Briefly, the cultures were centrifuged at 4,000 rpm in a bench centrifuge for 15 minutes to sediment the organisms. The pellet was resuspended in lysing buffer containing 300 microlitres (µl) of 0.1 molar (M) Tris pH 8.0; 50 µl of 10% sodium dodecyl sulfate (SDS). The mixture was incubated at 37 ^{0}C for 30 minutes (min) and then at 56 ^{0}C for 30 min. The mixture was then transferred to 1.5 ml eppendorf tube and extracted with an equal volume of phenol/chloroform/isoamyl alcohol at a ratio of 25:24:1, respectively. The supernatant was transferred into a clean sterile tube and

extracted with chloroform/isoamyl alcohol (24:1). Adding 2 volumes of absolute ethanol precipitated the extracted DNA in the aqueous phase. The extracted DNA was washed in absolute alcohol, vacuumed dried and dissolved in 50 µl of distilled water. Five microlitres of the resuspended DNA was used in the PCR-based RAPD amplification technique.

Arbitrary primers. A pair of arbitrary oligonucleotide primers was designed and used for random RAPD-PCR amplification (Table 1). Each pair of arbitrary primers would be expected to anneal to the sites to which they are matched or partially matched. The oligonucleotide sequences of the primers are shown (Table 1). All primers were synthesized on a DNA synthesizer (Milliigen/Biosearch, a division of Millipore Burlington, MA, USA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, VA, U.S.A.)

*Randomly amplified polymorphic DNA (RAPD)***.** A stock buffered solution containing 250 µl of 10× PCR buffer, 12.5 µl at a concentration of 125 millimole (mM) for each dATP, dTTP, dGTP and dCTP, and 100 µl of 25 mM Mg Cl_2 was prepared in a 1.5 ml eppendorf tube. The primers were used at a concentration of 20 picograms, and double distilled water was added to bring the volume of the stock buffer solution to 1.5 ml. A volume of 5.0 µl of the target DNA was added to 44 µl of the stock solution in PCR tubes and mixed by vortexing. A volume of 1.0 µl of Taq DNA polymerase (Perkin Elmer, Amersham, USA) was used at a concentration of 5.0 units. To avoid evaporation of the PCR mixture during the high temperature of the thermal cycling profiles, a drop of mineral oil was added to cover the reaction mixture. All PCR amplification reactions were carried out in a final volume of 50 µl. The thermal cycling profiles were as follows: a 2-min incubation at 95 ^{0}C, followed by 40 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 30 sec and 72 ^{0}C for 45 sec, and a final incubation at 72 ^{0}C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, N.J. U.S.A.) Following amplification, 20 µl from each PCR reaction containing amplified product were loaded onto gels of 1.5% SeaKem agarose (FMC Bioproduct, Rockland ME., U.S.A.) and electrophoresed. The gels were stained with ethidium bromide and the PCR products were identified following visualization under UV light.

Results

In this study, random primers were used to prime DNA synthesis. We were able to report on DNA sequence diversity among Sudanese strains of *H. somnus* strains using a pair of arbitrary primers. The pair of primers was able to produce a different pattern of amplification from the five strains *H. somnus*. The primers amplified the five strains of the organism forming 2 to 7 bands of varying size. This result indicated that random pattern of amplification. Lanes 2, 3, 4, 5 and 6 showed 5, 6, 2, 4, and 4 bands, respectively. This pattern of amplification indicates a great genetic diversity among the five strains of *H. somnus* used in this study. Lane 4 and 5 showed 2 bands of similar molecular weight but higher than that of the DNA marker.

The negative control samples contain all components of the PCR mixture except the DNA target from *H. somnus.* No amplification product was produced in lane 6, which represents the negative control sample (Fig. 1).

Table 1. DNA sequences and sources of arbitrary primers used in PCR-based RAPD fingerprinting

Primer	Sequence	Oligonucleotide size
1. P1 (5)	AGGACGCAGAAATAGCAGTA	20 bases
2. P2 (5)	ATTTAATTGAGTGGCGTGAG	20 bases

Discussion

A PCR-based DNA fingerprinting method, termed randomly amplified polymorhpic DNA (RAPD) profiles, was developed by WELSH and McCLELLAND (1990) and WILLIAMS et al. (1990). In this method they used a single randomly generated primer of 10 bases in length or longer to prime and differentiate DNAs from various sources. Other workers optimized this single primer PCR-based RAPD technique for detection of genetic variation among bacterial isolates belonging to the same species (MAZURIER et al., 1992; FAYOS et al., 1992; LAM et al., 1995). Recently, instead of a single oligonucleotide primer, a pair of randomly generated primers was evaluated for random PCR amplification (GIESENDORF et al., 1993).

In the present study, we describe the molecular characterization of *H. somnus* for the first time in the Sudan. This pair of primers was able to amplify the strains *H. somnus*. The primers amplified the five strains of the organism forming 2 to 7 bands of varying size, which indicated random pattern of amplification. This pattern of amplification indicates a genetic heterogeneity between the five strains of *H. somnus*. Lanes 4 and 5 showed 2 bands of similar molecular weight but higher than that of the DNA marker. This finding indicated that the random primers amplified PCR products of a very large base pair (bp) size from the whole genome of the organism. The specificity studies indicated that the random primers failed to produce amplification product from the negative control sample (Fig. 1).

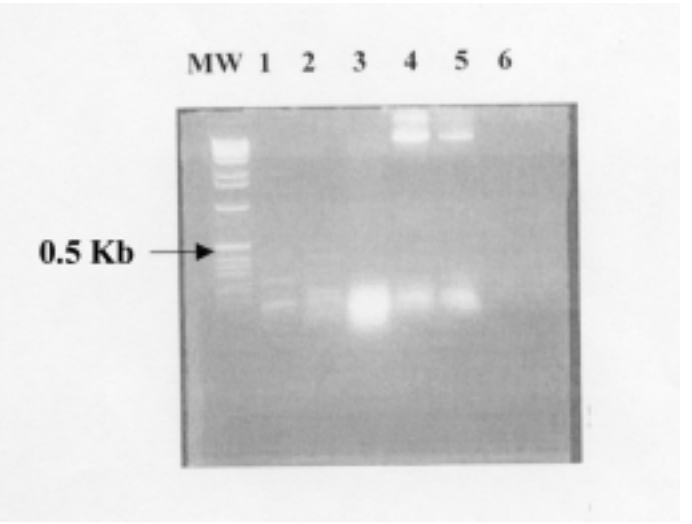

Fig. 1. PCR-based RAPD fingerprinting using a pair of arbitrary primers. Lane MW: molecular weight marker; Lane 1-5: whole DNA extracted from different strains of *H. somnus*

In the present study, bands of the same size (bp) were observed among the five strains of *H. somnus*. It is generally recognized that bands of the same size may indicate homology among isolates of *Cambylobacter jejuni* (WELSH and McCLELLAND, 1990; LAM et al., 1995). However, it is well documented that PCR products of the same size are not necessarily to be of the same DNA sequences. Therefore, these bands may have different

DNA sequences, which indicate different genetic make up (ARADAIB et al., 2003). Thus, DNA sequencing technique would be necessary to confirm the specificity of the nucleotide sequences of similar bands.

In conclusion, the scientific data presented in this paper indicates that application of PCR-based RAPD fingerprinting using whole DNA and arbitrary primers would provide a rapid and sensitive method for detection of genetic variations among different isolates of *H. somnus*.

References

ANGEN, A. (1998): Detection of *Haemophilus somnus* by polymerase chain reaction. Vet. Microbiol. 59, 203-211.

APPUHAMY, S., R. PARTON, J. G. COOTE, H. A . GIBBS (1997): Genomic fingerprinting of *Haemophilus somnus* by a combination of PCR methods. J. Clin. Microbiol. 35, 288-291.

ARADAIB, I. E., B. ABBAS (1985): A retrospective study of diseases diagnosed at the University of Khartoum Veterinary Medical Teaching Hospital. Sud. J. Vet. Sci. Anim. Husb 24, 55-66.

ARADAIB, I. E.; W. S. SMITH, J. S. CULLOR, B. I. OSBURN (2003): A multiplex PCR for simultaneous detection and differentiation of North American serotypes of bluetongue and epizootic hemorrhagic disease viruses. Comp. Immunol. Microbiol. Infec. Dis. 26, 77-87.

DERKS, R. E., S. A. ANNA, R. C. DILMAN (1973): Epizootiology and pathogenesis of *Haemophilus somnus* infection. Am. J. Vet. Med. Assoc. 163, 866-869.

FAYOS, A., R. J. OWENS, M. DESAI, J. HERNADEZ (1992): Ribosomal RNA gene restriction fragment diversity among Lior biotypes and penner serotypes of *Campylobacter jejun*i and *C. coli*. FEMS Microbiol. Letters 95, 87-94.

GIESENDORF, B. A. J., A. VAN-BEKUM, A. KOEKEN, H. STIGMAN, M. H. C. HENKENS, J. H. GOOSSENSH. M. G. NISTERSVAN, W. G. V. QUNIT (1993): Development of species-specific DNA probes for *Campylobacter jejuni*, *C. coli* and *C. lari* by polymerase chain reaction fingerprinting G. Clin. Microbiol. 31 1541-1546.

INZANA, T. G., J. HENSLEY, J. MCQUISTON, A. J. LESSE, A. A. CAMPAGNARI, C. M. BOYLE, M. A. APICELLA (1997): Phase variation and conservation of lipooligosaccharide epitopes in *Haemophilus somnus.* Infect. Immun. 11, 4675-4681.

LAM, L. M., R. YAMAMOTO, A. J. MASSA (1995): DNA diversity among isolates of *Campylobacter jejuni* detected by PCR-based fingerprinting. Vet. Microbiol. 45, 269-274.

MAZURIER, S., V. A. GIESSEN, K. HEUVELMAN, K. WERNARS (1992): RAPD analysis of Cambylobacter isolates: DNA fingerprimting without the need to purify DNA. Letters Applied Microbiol. 14, 260-262.

SHIGIDI, M.T. A., A. B. HOERLEIN (1970): Characterization of the Haemophilus- like organism of infectious thromboembolic meningoencephalitis of cattle. Am. J. Vet. Res. 31, 1017-122.

WELSH, J., M. McCLELLAND (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res. 18, 7213-7228.

WILLIAMS, J. G., A. R. KUBLECIK, K. J. LIWAK, J. A. RAFASKI, S. V. TINGGEY (1990): DNA polymorphism amplified by arbitrary primers are useful genetic markers, Nucleic Acid Res. 18, 6531-6535.

Received: 1 March 2003
Accepted: 7 November 2003

ELTOUM, K., I. ARADAIB, S. EL-SANOUSI: Fingerprinting analiza polimorfne DNA amplificirane pomoću lančane reakcije polimerazom za dokaz genetičke raznolikosti sudanskih izolata bakterije *Haemophilus somnus*. Vet. arhiv 73, 315-321, 2003.

SAŽETAK

Haemophilus somnus se često smatra uzročnikom infekcijskog tromboemboličnog meningoencefalitisa u goveda. Pet sojeva te bakterije bilo je izdvojeno iz klinički oboljelih goveda. U Sudanu se javlja klinički sindrom uzrokovan tom bakterijom, a očituje se nervnim znakovima. Izolati uzročnika bili su međusobno različiti po morfološkim i serološkim značajkama. Arbitrarne oligonukleotidne početnice rabljene su za umnažanje bakterijske polimorfne DNA pomoću lančane reakcije polimerazom.Tom je metodom dokazana heterogenost umnoženih DNA sojeva *Haemophilus somnus*. Rezultati upućuju na zaključak da se nasumce umnožena polimorfna DNA pomoću lančane rekacije polimerazom može upotrijebiti za proučavanje molekularne epidemiologije bakterije *H. somnus* izdvojene iz prirodno inficiranih goveda.

Ključne riječi: *Haemophilus somnus*, nasumce umnožena polimorfna DNA, Sudan