J. Protozool. Res. 14, 32-36 (2004)

BABESIA CABALLI: ANALYSIS OF CHROMOSOMES SEPARATED BY PULSE FIELD GEL ELECTROPHORESIS

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Key words: *Babesia caballi*; Pulsed field gel electrophoresis; Chromosome; Rhoptry gene

ABSTRACT

Pulsed field gel electrophoresis of intact chromosomes of *Babesia caballi* revealed 4 chromosomes in the genome. The genome size of *B. caballi* was estimated to be 8.9 megabase (Mb). The sizes of chromosomes 1, 2, 3 and 4 were estimated to be 0.9, 1.1, 2.8 and 4.1 Mb, respectively. The *bc48* rhoptry gene probe, derived from *B. caballi,* hybridized only to chromosome 3 (2.8 Mb).

INTRODUCTION

Babesia caballi is an economically important haematozoon, which is transmitted to horses by ticks in tropical and subtropical regions of the world (Friedhoff 1982; Schein 1988). Because *Babesia* organisms do not undergo a stage of chromosomal condensation during division (Rudzinska 1981), karyotyping requires DNA pulsed field gradient gel electrophoresis (PFGE), which has been used successfully to resolve that *Babesia bovis* and *Babesia bigemina* have 4 chromosomes in the haploid genome (Ray et al. 1992; Jones et al. 1997). Recently, we isolated a clone, bc48 gene (GenBank Accession No. AB017700) (Ikadai, Xuan et al. 1999), from a cDNA expression library using the monoclonal antibody BC11D (Ikadai, Tamaki et al. 1999). Although this clone had more than 2 copies in the *B. caballi* genome, it gave a complex Southern blotting pattern. In this study, we investigated the chromosomes of *B. caballi* by using PFGE to separate intact chromosomes and chromosomes that had been hybridized with a bc48 gene.

MATERIALS AND METHODS

The USDA strain of *B. caballi* was used. The *in vitro* culture condition was the same as described previously (Avarzed et al. 1997). *B. caballi*-infected horse erythrocyte from cultures were isolated using the Percoll gradient method (Bhushan et al. 1991). Cells were resuspended in phosphate-buffered saline (PBS) at approximately 8 x 10^8 organisms/ml and added to an equal volume of 2.0% Chromosomal Grade Agarose (Bio-Rad Laboratories, California) in PBS. The mixture was transferred by pipette into 1.5 x 10 x

5 mm molds and allowed to gel at 4 ^{0}C. Solidified agarose blocks were expelled into lysis buffer (0.2 M NaCl, 10 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1% sodium dodecyl sulfate (SDS), containing 1 mg/ml protease K), incubated for 72 hr at 55 C, and then stored in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH8.0) at 4 ^{0}C.

PFGE was performed using a clamped homogeneous electric field (CHEF) apparatus (Bio-Rad). Chromosome-containing blocks were inserted into wells in a 1% (w/v) agarose gel-TAE buffer (40 mM Tris-base, 40 mM acetic acid, 10 mM EDTA pH 8.0). Electrophoresis was performed at 14 ^{0}C, at constant voltage. Two different PFGE programs were used to separate the intact putative chromosomes of B. caballi, as follows: (A) The first stage of electrophoresis was performed for 24 hr at 2 V/cm with 1,200 sec pulse time at an angle of 96°. The second stage of electrophoresis was performed for 24 hr at 2 V/cm with 1,500 sec pulse time at an angle of 100°. The final stage of electrophoresis was performed for 24 hr at 2 V/cm with 1,800 sec pulse time at an angle of 106°; and (B) The first stage of electrophoresis was performed for 36 hr at 3 V/cm with 400 sec pulse time at an angle of 106°. The second stage of electrophoresis was performed for 24 hr at 2 V/cm with 1,500 sec pulse time at an angle of 100°. The final stage of electrophoresis was performed for 12 hr at 2 V/cm with 1,800 sec pulse time at an angle of 106°. The following chromosome size markers were used: *Schizosaccharomyces pombe* (Bio-Rad) with chromosomes of 3.5, 4.6 and 5.7 megabase (Mb); and *Saccharomyces cerevisiae* (Bio-Rad) with chromosomes ranging from 0.2 to 2.2 Mb. Gels were stained with ethidium bromide (0.5 μg/ml in TAE buffer) for 1 h, destained in distilled water for 30 min and photographed under ultraviolet transillumination. Following gel depurination (0.25 M HCl), denaturation (0.5 M NaOH, 0.6 M NaCl) and neutralization (1.0 M Tris-HCl pH 7.0, 0.6 M NaCl), DNA bands were transferred overnight to Hybond-N nylon membranes (Amersham Biosciences Ltd., U.K.) using 20 x SSC (3 M NaCl, 0.3 M trisodium citrate), and hybridized with a [^{32}P]-labeled probe derived from the *bc48* gene by using the random primer DNA synthesis method in the presence of [^{32}P]-dCTP (Amersham) (Feinberg and Vogelstein, 1983). Prehybridization and hybridization were performed overnight at 42 C. Membranes were washed 3 times with 0.1 x SSC containing 0.1% SDS at 42 C for 15 min. Bands hybridizing to the probe were detected by standard techniques.

RESULTS AND DISCUSSION

PFGE of the genomic DNA of the USDA strain of *B. caballi* resulted in 4 bands (Fig. 1A). The 2 largest putative chromosomes did not always separate and often appeared as 1 band of approximately 3.4 Mb (Fig. 1B). This 3.4 Mb chromosome band seemed brighter than other chromosomes, such as the 1.1 Mb and 0.9 Mb bands (Fig. 1B). Moreover, the third and fourth chromosomes in *B. bovis* often migrated together and appeared as a single bright chromosome band that migrated slower than the actual chromosome 3 alone (Jones et al. 1997). Therefore, *B. caballi* has 4, and not 3, chromosomes. The 2 largest bands were estimated to be 4.1 Mb and 2.8 Mb, and a comparison of the 2 smaller bands indicated that they were 1.1 Mb and 0.9 Mb in size. On the basis of these 4 bands, we estimated the genome size of *B. caballi* to be 8.9 Mb. To our knowledge, this is the first report of number of the *B. caballi's* chromosomes.

These PFGE experiments provide evidence that the genome of *B. caballi* has 4 chromosomes.

Using PFGE Ray et al. (1992) and Jones et al. (1997) found that *B. bovis* and B. bigemina have 4 chromosomes. Jones et al. (1997) suggested that the genome of *B. bovis* is approximately 9.4 Mb in size, and that the genomes of the Mexican and Costa Rican isolates of *B. bigemina* are 10 Mb and 10.8 Mb, respectively. The *Babesia* organism's genome was located very close to, and in the identical mobility zone as, *B. caballi, B. bovis,* and *B. bigemina* chromosomes. Therefore, chromosomal polymorphism is suggested by the variation in the size of chromosome migration patterns in the *B. bigemina* isolates Mexico and Costa Rica (Ray et al. 1992). From these findings, we suggest that *B. caballi* might also exhibit size variability between different isolate.

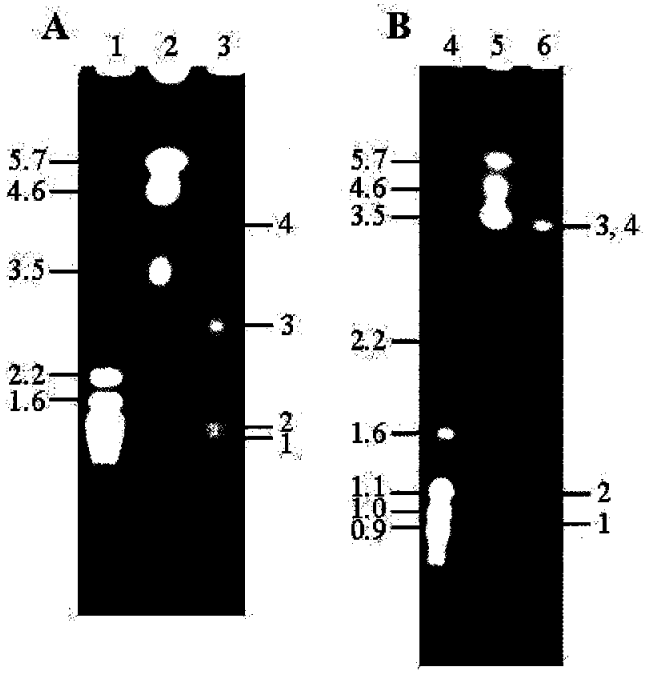

Fig. 1 PFGE separation of the chromosomal DNA of *B. caballi*. PFGE parameters were: (A); pulse time, 1,200 sec; electric field strength, 2 V/cm; running time, 24hr; angle, 96°; followed by pulse time, 1,500 sec; electric field strength, 2 V/cm; running time, 24hr; angle, 100°; next followed by pulse time, 1,800 sec; electric field strength, 2 V/cm; running time, 24hr; angle, 106°; (B) pulse time, 400 sec; electric field strength, 3 V/cm; running time, 36hr; angle, 106°; followed by pulse time, 1,500 sec; electric field strength, 2 V/cm; running time, 24 hr; angle, 100°; next followed by pulse time, 1,800 sec; electric field strength, 2 V/cm; running time, 12 hr; angle, 106°. *S. cerevisiae* chromosomes (lanes 1 and 4) and *S. pombe* chromosomes (lanes 2 and 5) were used as a size markers. *B. caballi* chromosome (lanes 3 and 6) numbers are always on the right of the figure.

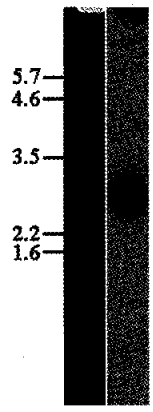

Fig 2 Southern hybridization of [^{32}P]-labeled *bc48* cDNA probe to *B. caballi* chromosomes. The PFGE parameters on the left were the same as in Fig. 1A.

The *bc48* gene probe hybridized only to chromosome 3 (2.8 Mb), which indicates that the third chromosome contains all copies of the *bc48* gene in our experiments (Fig. 2).

The *bc48* gene contained more than 2 copies, as a multicopy gene, in the *B. caballi* genome (Ikadai, Xuan et al. 1999); therefore, our results indicate that the third chromosome contains all sets of the bc48 gene.
Recognition of the value of a physical analysis of genomes has led to the creation of consortia of laboratories generating data and resource genetic material from a range of parasites that impact on animal health. Further support for 4 chromosomes in the genome of *B. caballi* comes from the studies using the other B. caballi DNA hybridizing probes for the construction of a physical linkage map of *B. caballi* chromosomes.

In conclusion, our experiments indicate that *B. caballi* has 4 chromosomes, a genome of 8.9 Mb and that the rhoptry associated protein gene, *bc48,* is on chromosome 3.

ACKNOWLEDGMENTS

This study was supported by Grant-in-Aid for Young Scientists from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, and the Kitasato University Research Grant for Young Researchers and Encouragement of Young Scientists.

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