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THE GENOME OF ANAPLASMA: DNA BASE COMPOSITION AND DNA/DNA HYBRIDIZATION

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ABSTRACT

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The Tm value of DNA from *Anaplasma centrale* and *Anaplasma marginale* was found to be 87,1 °C and 89,3 °C, respectively. The G + C content, calculated from the Tm, was 45,1 % for *A. centrale* and 48,5 % for *A. marginale*. Identical hybridization patterns were obtained when the DNA from one species was hybridized to restriction endonuclease-digested DNA from the other species.

INTRODUCTION

Anaplasmosis, a tick-borne, haemolytic disease of cattle which occurs worldwide, is caused by *Anaplasma marginale* and *Anaplasma centrale*. After natural infection, *A. marginale* organisms are seen in the host erythrocytes within 3–6 weeks. An acute phase of high parasitaemia occurs within the following 4–9 days (Ristic, 1977). This is followed by a chronic phase during which a low parasitaemia may persist indefinitely.

Anaplasmosis is controlled immunoprophylactically by using live, attenuated *A. marginale* parasites (Ristic, Sibinovic & Welter, 1968), heat-killed *A. marginale* (Ristic *et al.* 1968) or premunization with *A. marginale* or *A. centrale* (Norman, 1973). The problems experienced with all these forms of vaccination include variable protection, isoerythrolysis, reversion to virulence and diminished milk production in lactating cows (Norman, 1973).

Rapidly developing genetic engineering techniques allow for novel approaches to vaccine production. Their successful application depends on a knowledge of the physical parameters of the DNA studied which, in the case of *Anaplasma*, is almost non-existent. To date, there have been only 2 reports dealing with the physicochemical properties of *A. marginale* DNA (Senitzer, Dimopoullos, Brinkley & Mandel, 1972; Ellender & Dimopoullos, 1967). The latter workers suggested that the *A. marginale* genome consists of single-stranded DNA, while Senitzer *et al.* (1972) found that this organism contains double-stranded DNA with a G + C content of 51 %. This lack of information on the genomes of these parasites prompted us to investigate whether there were differences between *A. centrale* and *A. marginale* at the DNA level.

MATERIALS AND METHOS

DNA Isolation. Blood from Anaplasma-infected cattle was centrifuged at 900 × g for 15 min for the removal of the buffy coat. After 3 cycles of centrifugation/buffy coat removal, the red blood cells (RBC) were passed through a Whatman CF-11 column (Richards & Williams, 1972; Ambrosio, Potgieter & Nel, 1986). Infected RBC were lysed by incubation in 10 % SDS in 10 mM Tris-HC1 buffer, pH 7,5, and 0,1 M EDTA for 15 min at 37 °C. Lysates were digested with Proteinase K (100 μg mℓ$^{-1}$[1]), for 1 h at 37°C. DNA was then extracted 3 times with an equal volume of phenol and twice with an equal volume of chloroform:octanol (24:1, v/v). After ethanol precipitation, DNA was resuspended in 10 mM Tris-HC1, pH 7,5, 1 mM EDTA and purified further by cesium chloride/ethidium bromide density gradient centrifugation.

Tm determination. Purified *Anaplasma* DNA was dissolved in 1× SSC (0,15 M NaC1, 0,015 M sodium citrate) and melted at a linear temperature increase rate of 60 °C/h. Melting points (Tm) were calculated by plotting A_{260} values, determined in a Beckman DU-8 spectrophotometer, against temperature.

DNA restriction and DNA/DNA hybridization. Restriction enzymes *Bam* HI, *Hind* III, *Cla* I and *Hinf* I were obtained from Anglian biotechnology[2]. Restrictions were performed at 37 °C in a buffer solution as recommended by the manufacturer. Digested DNA (± 15 μg) was electrophoresed through a 0,8 % agarose gel and transferred to a nylon membrane[3] (Southern, 1975). The blots were hybridized with ^{32}P-labelled DNA (Southern, 1975) from *A. marginale* or *A. centrale*. Autoradiographs were exposed for 2 days at −70 °C, using Cronex MRF-31 X-Ray film with a lightning plus intensifying screen[4].

RESULTS AND DISCUSSION

Melting properties of A. centrale *and* A. marginale *DNA*. A commonly used technique for determining the DNA base composition is based on the linear increase in both density and thermal stability of DNA, with an increase in the G + C content (Marmur & Doty, 1972.; Schildkraut, Marmur & Doty, 1962). These 2 parameters were used to calculate the base composition of *A. centrale* and *A. marginale* DNA. In addition to being a paramenter that can be used for calculating base composition, thermal stability of DNA could indicate the degree of intra-molecular heterogeneity of the molecule (Marmur & Doty, 1972). The melting points of *A. centrale* (a Tm of 87,1 °C) and *A. marginale* (A Tm of 89,3 °C) DNA in 1× SSC are shown in Fig. 1. The G + C content calculated from these Tm values, using the equation of De Ley (1970), is 45,1 % for *A. centrale* and 48,5 % for *A. marginale*.

DNA/DNA hybridization. *A. centrale* and *A. marginale* DNA were digested with *Hind* III, *Cla* I, *Hinf* I and *Bam* HI, electrophoresed, and transferred to nylon membranes. Undigested *A. centrale* and *A. marginale* DNA was ^{32}P-labelled and used as probes. Restricted bovine DNA was used as a control to determine the amount of bovine DNA background in the blots (Fig. 2, lane e). No differences in hybridization patterns were detected between *A. centrale* and *A. marginale* (Fig. 2 & 3). There was a variation, however, in the intensity of the hybridization bands obtained. In Fig. 2 the intensity of the *A. marginale* is higher than *A. centrale* using a *A.*

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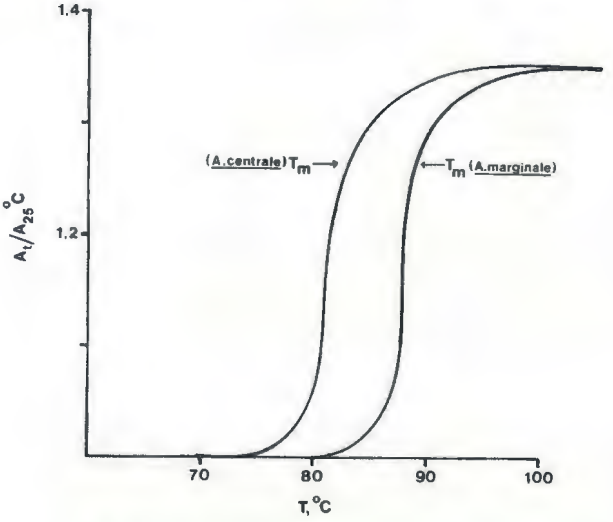

FIG. 1 Thermal denaturation of *A. marginale* and *A. centrale* DNA. 20 μg of purified DNA was analysed. Denaturation was in 1× SSC as described in Material and Methods

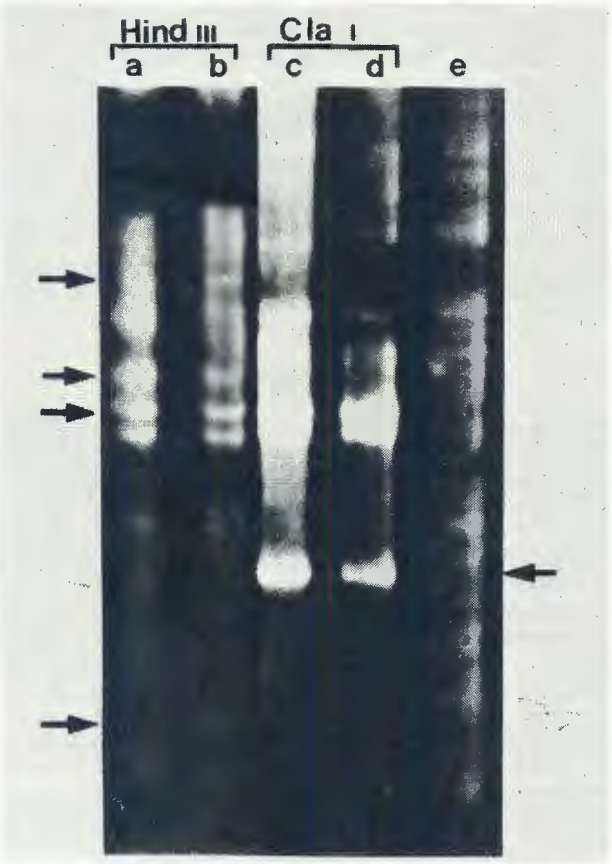

FIG. 2 Southern blot analysis of DNA from *A. marginale* and *A. centrale* digested with *Hind* III and *Cla* I. Lanes a and c, *A. marginale* DNA and lanes b and d, *A. centrale* DNA. Lane e is *Hind* III-digested DNA from an uninfected bovine. The arrows indicate bands of *Anaplasma* DNA hybridizing to *A. marginale* DNA probe. DNA was isolated as described in Materials and Methods

marginale probe. In Fig. 3a, the intensity of the *A. centrale* bands is approximately 2× that of the *A. marginale* bands obtained with a *A. centrale* probe. Equal amounts of DNA were loaded in each lane (see Materials and Methods).

The G + C content of 48,5 % we obtained for *A. marginale* differs from that of the 51 % reported by Senitzer *et al.* (1972). This discrepancy between the

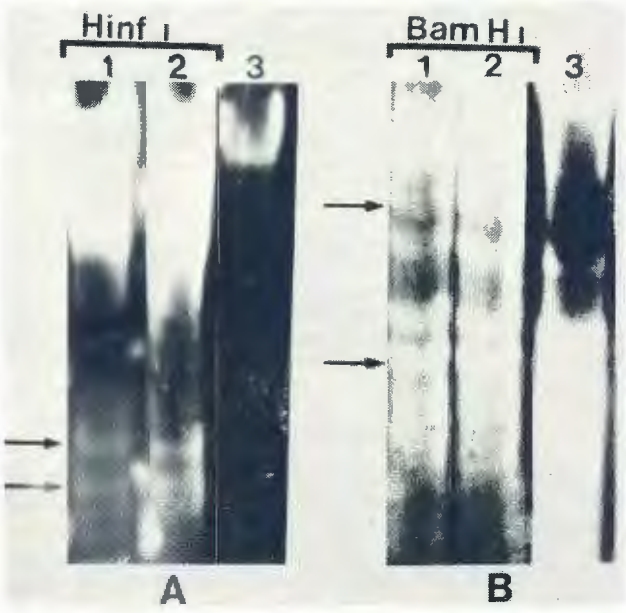

FIG. 3 Hybridization of *Hinf* I and *Bam* HI-digested *Anaplasma* DNA to *A. centrale* DNA probe. Lane 1 (A & B), *A. marginale* DNA (15 μg); Lane 2, (A & B), *A. centrale* DNA (15 μg); Lane 3, *Hinf* I (A) and *Bam* HI (B)-digested bovine DNA (15 μg). Arrows indicate hybridization bands common to both *Anaplasma* species

results is probably due to a difference in purity of the parasite preparations used to isolate the DNA and the method used to obtain the G + C value (Senitzer *et al.* 1972). DNA used in this study was prepared from parasites obtained from RBC carefully deprived of leucocytes (Ambrosio *et al.* 1986). The G + C content of *A. centrale* had not previously been determined.

The determination of DNA sequences of homologous regions for a number of species commonly disclose many nucleotide substitutions, and the pattern of rearrangements can be used to establish the relatedness of the species. Closely related species, such as man and chimpanzee, differ by only 2 % in their nuclear DNA sequences (Britten, 1986). This implies about 60 million sequence differences. Most of these differences have little or no effect on phenotype. No differences were detected in the DNA/DNA hybridization patterns of *A. centrale* and *A. marginale*. This result does not exclude the possibility of differences in the base sequence of the DNA, but merely reflects extensive regions of homology. DNA sequence changes (deletions, substitutions, rearrangements and insertions) are the most likely source of phenotypic variation in evolution. They can affect the regulation of gene expression and thereby influence development and morphology. Phenotypic differences observed between the 2 species of *Anaplasma* could therefore possibly be caused by such sequence changes not reflected in the hybridization patterns.

Interspecies comparison of DNA alone cannot be used to establish differences between species (Britten, 1986). Coding sequences make up only a small part of the total DNA. In *Anaplasma*, at this stage, we do not know what the function of most of the DNA is, and the significance of our results for the classification of the 2 species remains uncertain.

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