

EXTRACHROMOSOMAL NUCLEIC ACIDS IN BOVINE BABESIA

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Two kinds of small extrachromosomal nucleic acid elements were found in the bovine babesias, Babesia bovis and B. bigemina. One element with an apparent size of 5.5 kilobase pairs (kbp) is a double stranded RNA related to virus like particles. Another molecule is a double stranded DNA with a molecular size of about 6.2 kbp. Southern blot comparison of restriction DNA fragments of the latter molecule, which is present in both B. bovis and B. bigemina is described.

Key words: *Babesia bovis* – *Babesia bigemina* – mitochondrial DNA – DNA element

A conserved extrachromosomal DNA element of about 6.2 kb has been observed in various members of the phylum Apicomplexa represented by species of the genera *Plasmodium*, *Babesia*, *Eimeria*, *Toxoplasma*, and *Theileria* (Joseph et al., 1989; Hall et al., 1990). The 6.2 kb DNA element of *P. gallinaceum* has sequences homologous to genes encoding the polypeptide cytochrome b and the large mitochondrial rRNA, suggesting it to be a mitochondrial episome (Aldritt et al., 1989). It can thus be classified as an organelle DNA (Rush & Misra, 1985). By analyzing total nucleic acids of *B. bovis* in agarose gel electrophoresis we also found a 6.2 kb DNA element, as well as a 5.5 kb dsRNA which was correlated to virus-like particles (Johnston et al., 1991). The element is present in different isolates of *B. bovis* as well as in various isolates of another bovine *Babesia* prevalent in Brazil, the *B. bigemina*. Furthermore, restriction enzyme analyses show that the element is conserved but has interspecies non-homologous sequences.

PRESENCE OF THE 6.2 KB DNA ELEMENT IN ISOLATES OF *B. BOVIS* AND *B. BIGEMINA*

The 6.2 kb DNA element can be detected by electroporesis in agarose gels stained with

ethidium bromide of genomic DNA of isolates of *B. bovis* and *B. bigemina*, as a band running below the 6.6 kb DNA marker of lambda *Hind*III (Fig. 1A lanes 1 and 2; Johnston et al., 1991). This band is not detected in genomic DNA preparations of bovine leucocytes (not shown). Southern blot analyses, using cloned *Eco*RI fragment of the DNA element of *B. bovis* as probe, show that it is conserved and present in various isolates of *B. bovis* as well as of *B. bigemina* (results not shown).

RESTRICTION PATTERN DIFFERENCES BETWEEN THE 6.2 KB DNA ELEMENT OF *B. BOVIS* AND *B. BIGEMINA*

To determine the extent of homology between the elements of the two species of *Babesia*, genomic DNA of *B. bovis* and *B. bigemina* were cut with restriction enzymes and Southern blotted, using the nick-translated DNA element of *B. bovis*, purified from agarose gel, as probe. The restriction pattern of the 6.2 kb DNA from both species is similar when it is cut with *Hind*III (Fig. 1B, lanes 5 and 6). However, when cut with *Eco*RI or *Cla*I, the pattern observed is different. The 6.2 kb DNA of *B. bovis* produced two fragments of 4.4 and 1.8 kb when cut with *Cla*I and four fragments of 2.6, 1.9, 1.1 and 0.6 kb when cut with *Eco*RI (Fig. 1, lanes 3 and 7). The 6.2 kb DNA of *B. bigemina* is cut by *Cla*I producing a visible fragment of 6.0 kb (Fig. 1B, lane 8). With *Eco*RI the fragments produced are 2.9, 1.9, 0.6, 0.45 and 0.35 kb in size (Fig. 1B, lane 4). The sum of the

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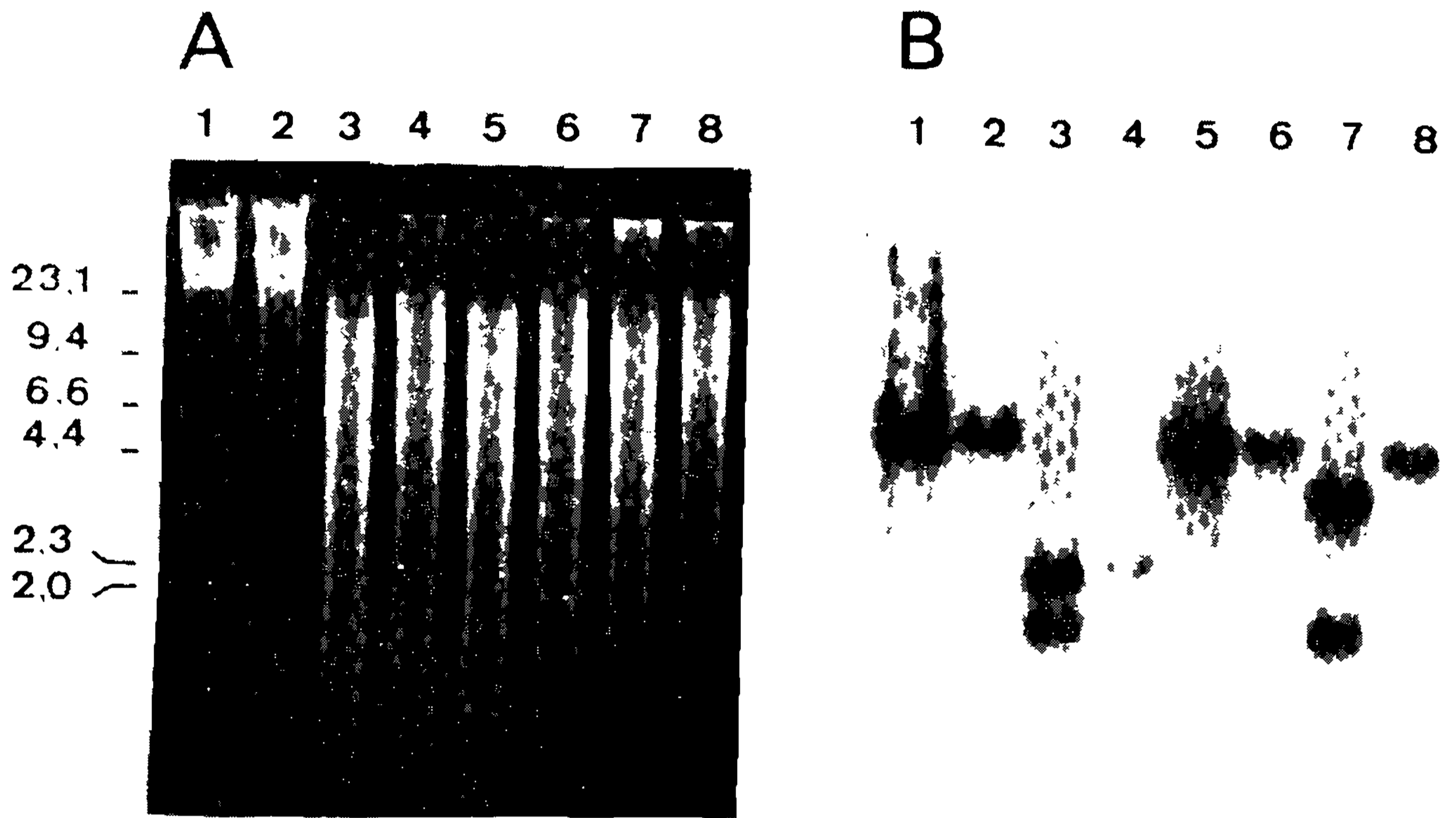


Fig. 1: restriction enzyme analysis of the 6.2 kb DNA of bovine *Babesia*. Agarose gel (0.7%) electrophoresis of genomic DNA of *Babesia* spp cleaved with various restriction enzymes was stained with ethidium bromide (A) or Southern blotted (Maniatis et al., 1982) using the 6.2 kb DNA as probe (B). DNA of *B. bovis* (lanes 1,3,5 and 7) and *B. bigemina* (lanes 2,4,6 and 8) were cut with *EcoRI* (lanes 3 and 4), *HindIII* (lanes 5 and 6) and *ClaI* (lanes 7 and 8) or uncut (lanes 1 and 2).

restriction fragments produced, in no case, is greater than 6.2 kb. Interestingly, a second band with a size of approximately 13 kb is detectable in the hybridization to the genomic DNA of *B. bovis* but not to that of *B. bigemina* (Fig. 1B, lanes 1 and 2 respectively). Other enzymes tested (results not shown) were *PvuII*, *BglII*, *XbaI*, *SphI* and *BamHI*. None of them gave different restriction pattern between the 6.2 kb element of the two *Babesia* species.

CONCLUSION

We report the finding of a 6.2 kb DNA element present in different isolates of *B. bovis* as well as of *B. bigemina*, the ethiological agents of bovine babesiosis in the Americas. By restriction enzyme analyses and Southern blotting we found that it is conserved among the two species of *Babesia* although with some non-homologous sequences. Furthermore, the DNA element of bovine *Babesia* has sequences homologous to an extrachromosomal DNA element of a species of the genus *Theileria* (Morzaria, S., ILRAD, Kenya, personal communication in this Congress). The conservation of such element among all *Apicomplexa* members in which it was detected (Joseph et al.,

1989; Hall et al., 1990; Johnston et al., 1991) must still be demonstrated experimentally. The data to the present, however, is highly indicative of the conservation of this element pointing to a unique structural organization of the *Apicomplexa* suggested mitochondrial genome.

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