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# THE ISOLATION OF NUCLEIC ACID SEQUENCES SPECIFIC FOR *COWDRIA RUMINANTIUM*

S. C. WILKINS and R. E. AMBROSIO, Molecular Biology Section, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

WILKINS, S. C. & AMBROSIO, R. E., 1989. The isolation of nucleic acid sequences specific for *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research*, 56, 127–129 (1989).

Screening of a genomic library of *Cowdria ruminantium* has yielded twelve clones hybridizing to *Cowdria* DNA. These clones should be suitable for development into DNA probes specific for this organism.

## INTRODUCTION

Cowdriosis, or heartwater, is a tick borne disease of ruminants caused by the rickettsia-like parasite *Cowdria ruminantium*. The disease occurs in Africa and the Caribbean islands and poses a serious threat to livestock in the United States (Barré, Uilenberg & Morel, 1987) where potential vectors are present (Uilenberg, 1983), but do not harbour the disease (Uilenberg, 1982). Cowdriosis in the live animal cannot be diagnosed with certainty and unless treated is fatal (Provost & Bezuidenhout, 1987). Blood smears are of no value and serology is unreliable (Uilenberg, 1982; 1983).

The application of molecular techniques to the study of *Cowdria* has been severeley hampered by the low yields of organisms from infected animals. Until recently all attempts at cultivating the organism in cell culture have been unsuccessful due to a variety of factors. Recently, however, both the Welgevonden and Kwanyanga isolates of *C. ruminantium* have been successfully cultivated in a bovine endothelial cell line (Bezuidenhout, 1987; Bezuidenhout, Paterson & Barnard, 1985). With the objective of developing a rapid and sensitive diagnostic test for cowdriosis we have constructed a genomic library of the Welgevonden isolate of *in vitro*-cultured *C. ruminantium*. This paper presents the results of screening this library.

## MATERIALS AND METHODS

A genomic library of the Welgevonden isolate of *C. ruminantium* cultured *in vitro* in a bovine endothelial cell line (Bezuidenhout, 1985) was constructed in the expression vector λ*gt*11 as described by Hunyh, Young & Davis (1985). *Cowdria*-infected cells were lysed by sonication followed by addition of lysis buffer [10 mM Tris, pH 7,0, 0,25 mM EDTA, 1 % (w/v) SDS] and overnight incubation with 10 μg $m\ell^{-1}$ Pronase. *C. ruminantium* DNA was separated from host cell DNA by affinity chromatography (Dean, Johnson & Middle, 1985) using single stranded calf thymus DNA cellulose[1].

*Mycoplasma* DNA was from a culture obtained from Dr Trichardt, *Mycoplasma* Research, Onderstepoort. *Anaplasma centrale* DNA was from the red blood cells of an infected bovine and the sheep DNA was isolated from sheep buffy coat material.

Library screening was by plaque hybridization (Maniatis, Fritsch & Sambrook, 1982) using duplicate filters probed with labelled bovine DNA and total DNA from *Cowdria*-infected cells respectively.

Hybridization was at 65 °C for 16 h in a buffer as described by Maniatis *et al.* (1982). Blots were washed at high stringency in 0,1X SSC (1X SSC: 0,15 M NaC1, 0,015 M $Na_3$ citrate, pH 7,0) at 65 °C and autoradiographed overnight at −70 °C with intensifying screens. Nylon membranes were stripped and re-used by washing in 0,4 M NaOH for 120 min, followed by one wash in a buffer containing 0,1X SSC, 10 mM Tris pH 7,5, 0,1 % SDS.

## RESULTS AND DISCUSSION

It was not possible to differentially lyse the E5 cells to obtain pure *Cowdria* organisms. For this reason we isolated *Cowdria* DNA by affinity chromatography. The binding capacity of the cellulose was 3,5 mg $m\ell^{-1}$ and all detectable bovine DNA was removed by the column (Fig. 1). However, the yield

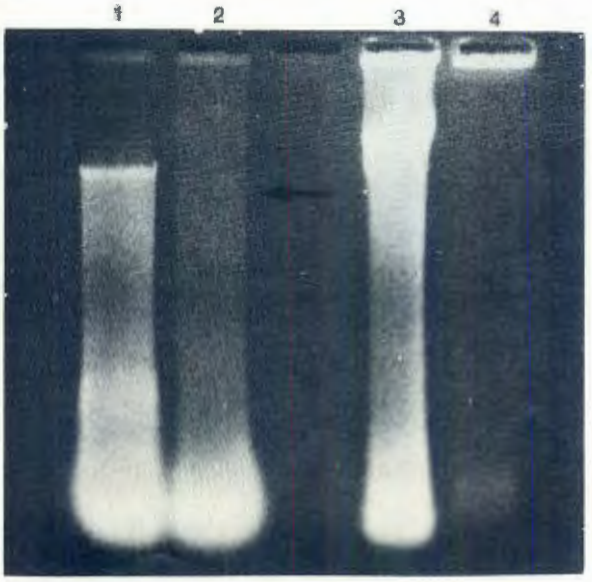


FIG. 1 Agarose gel electrophoresis of affinity column purified bovine and *Cowdria* DNA.

Lane 1: DNA from *Cowdria*-infected E5 cells: No column., Lane 2: DNA from *Cowdria*-infected E5 cells: Column purified., Lane 3: Uninfected bovine DNA (10 μg): No column., Lane 4: Uninfected bovine DNA: Column purified.

DNA was electrophoresed through a 0,7 % agarose gel at 60 V for 3 h. Arrow points to the position of the *Cowdria* DNA band.

[1] Sigma Chemical Co., St. Louis, Mo

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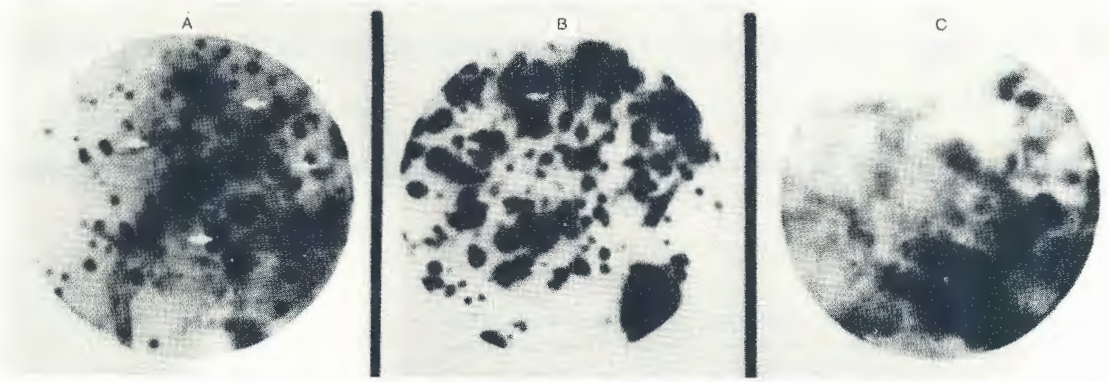


FIG. 2 Plaque hybridization to the *Cowdria* library.
A: Bovine DNA (uninfected cells) as probe. Arrows point to areas of no hybridization.
B: DNA from *Cowdria* infected cells as probe. Arrows point to areas of strong hybridization.
C: Bovine DNA (uninfected cells) as probe.

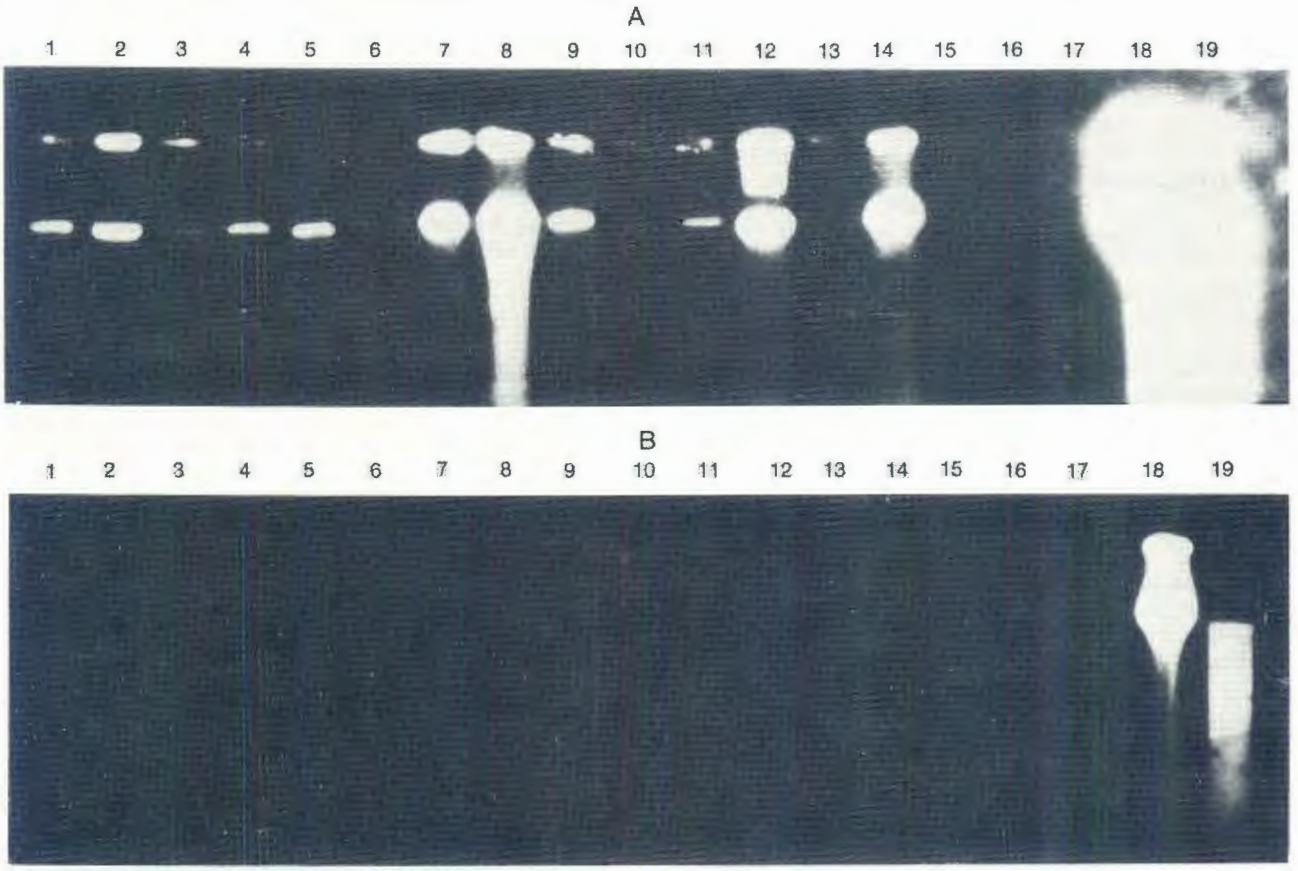


FIG. 3 Hybridization to southern blots of DNA from individual *Cowdria* DNA-containing plaques.
A: Probe was total DNA from *Cowdria*-infected cells. Lanes 1–17: DNA from recombinant plaques., Lane 18: Bovine DNA (uninfected)., Lane 19: *Cowdria*-infected E5 cell DNA.
B: Probe was bovine DNA. Lanes as in "A".

of *Cowdria* DNA was consistantly low, a problem which we have not been able to resolve. *Cowdria* DNA was digested with *Eco*R1, cloned into λ*gt*11, *in vitro* packaged and plated on a suitable host. A titer of $5 \times 10^7$ p.f.u. $m\ell^{-1}$ was obtained, of which 73 % were recombinants.

Library screening was by plaque hybridization using duplicate filters and labelled bovine DNA and DNA from *Cowdria*-infected cells as probes (Rigby, Dieckmann, Rhodes & Berg, 1977). Plaques that did not hybridize to the bovine DNA but hybridized to DNA from the infected E5 cells were considered to contain *Cowdria* specific sequences. Hybridization obtained in a typical screening experiment is shown in Fig. 2A, B & C.

Plaques were isolated from areas where no hybridization to bovine DNA occurred (Fig. 2A), but showed strong hybridization to DNA from the infected cells (Fig. 2B). These results were confirmed by once again purifying these plaques and again

hybridizing to DNA from uninfected bovine cells. No hybridization was obtained (Fig. 2C).

DNA was extracted from these purified plaques using a rapid procedure (Maniatis *et al.*, 1982) and hybridized to bovine DNA from uninfected cells as well as to *Cowdria* DNA. Of 800 plaques screened, 12 were obtained which consistantly hybridized to DNA from *Cowdria* infected cells and not to DNA from uninfected cells (see Fig. 3A & B). No cross-hybridization was obtained with DNA from any one of these plaques to *Mycoplasma, Anaplasma,* sheep or bovine DNA (not shown).

To date there has been only one report on the cloning of *C. ruminantium* DNA (Ambrosio, Du Plessis & Bezuidenhout, 1987). However, these genomic libraries contained a large proportion of host DNA and were difficult to screen. The value of a *Cowdria* library depends on the degree to which it represents the organism's genome. It was for this reason that care was taken to remove most of the host cell DNA prior to cloning into the expression vector in the experiments reported here. Twelve DNA sequences have been isolated from the library which are specific for DNA from *Cowdria*-infected cells. We intend to develop these sequences into diagnostic probes for *C. ruminantium* and to use them for epidemiological studies of this organism.

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