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HYBRIDIZATION OF DNA PROBES TO *A. MARGINALE* ISOLATES FROM DIFFERENT SOURCES AND DETECTION IN *DERMACENTOR ANDERSONI* TICKS

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ABSTRACT

AMBROSIO, R. E., VISSER, ELIZABETH S., KOEKHOVEN, YOLANDA & KOCAN, KATHERINE M., 1988. Hybridization of DNA probes to *A. marginale* isolates from different sources and detection in *Dermacentor andersoni* ticks. *Onderstepoort Journal of Veterinary Research*, 55, 227–229 (1988).

DNA from the Washington, South-Idaho, Virginia and Florida isolates of *Anaplasma marginale* was hybridized to probes specific for *Anaplasma centrale* and *A. marginale*. The *A. centrale* probes AC–2 and AC–4 hybridized to identical bands on all of these isolates. The hybridization pattern suggests that the Virginia, Florida and the South African isolates are similar. A number of bands were obtained with the Washington isolate which differed from those obtained with the other isolates. Probe AC–2 could be developed to identify relatedness among *Anaplasma* isolates. Probe AC–2 detected *A. marginale* DNA in midgut material from infected *Dermacentor andersoni* ticks. No hybridization was obtained with DNA from salivary gland tissues from these infected ticks.

INTRODUCTION

Anaplasmosis of cattle, caused by the rickettsial parasites *Anaplasma marginale* and *Anaplasma centrale* has the greatest worldwide prevalence among the tick-borne hemoparasitic diseases including protozoan diseases such as babesiosis and theileriosis (Smith, 1968). The less virulent *A. centrale* does not occur in the United States. This species causes a milder infection and cross-reacts serologically with *A. marginale*.

In the United States *A. marginale* is transmitted by ticks of the genus *Dermacentor*, of which 4 species have been identified as vectors (Kocan, 1986). In South Africa *A. centrale* has been shown to be transmitted by ticks of the genera *Hyalloma, Rhipicephalus* and *Boophilus* (Potgieter, 1979). *A. marginale* colonies have been demonstrated in tick midgut cells by light and electron microscopy (Kocan, 1986). Potgieter, Kocan, McNew & Ewing (1983) demonstrated colonies of *A. marginale* in the midgut of the tick, *Rhipicephalus simus*. *A. centrale* has not been identified in tick tissues (K. Kocan: Personal communication).

There are numerous examples of the use of DNA hybridization in the identification of parasites. Advantages of these methods include rapidity, ease of use, accuracy and sensitivity. These techniques are sensitive enough to identify different species as well as to detect parasites present in low numbers (Barker, Suebsaeng, Rooney, Alecrim, Dourado & Wirth, 1986).

Conventional techniques such as serology and microscopy are often not entirely suitable for the analysis of infected ticks, infection rates or the development of the parasite in the tick vector.

The isolation of *A. centrale* probes which also detect *A. marginale* has recently been reported (Visser & Ambrosio, 1987). In this paper we apply these probes to *A. marginale* isolates from different sources. The ability of these probes to detect *A. marginale* in *D. andersoni* ticks was also investigated.

MATERIALS AND METHODS

DNA isolation: A centrale and *A. marginale* (BW strain) DNA was isolated from infected erythrocytes as previously described (Visser & Ambrosio, 1987). DNA from the US-isolates of *A. marginale;* AM-Washington, AM-Florida, AM-South Idaho and AM-Virginia (Palmer, 1988) was isolated from stabilates using standard techniques (Maniatis, Fritsch & Sambrook, 1982).

Tick DNA was isolated from infected *D. andersoni* nymphs that had fed to repletion in 6 days, and were allowed to molt to adults. Control ticks were similarly fed on *A. marginale*-negative animals and processed identically. In total, one hundred infected ticks from each group were dissected and the mid-gut and salivary glands pooled, in aliquots representing material from 20 ticks each. DNA was extracted according to standard techniques (Maniatis *et al.*, 1982).

Restriction endonuclease digestion and DNA hybridization: DNA was digested to completion with *Eco*R1 (Amersham International) in a buffer as supplied by the manufacturer. Digested DNA was electrophoresed on an 0,8 % agarose gel and transferred to nylon membranes for hybridization (Southern, 1975).

Probes were labelled with ^{32}P-dCTP by nick-translation (Rigby, Dieckmann, Rhodes & Berg, 1977) to a specific activity of 10^8dpm μg^{-1}.

Hybridization was for 15 h at 65 °C in a solution containing 6X standard saline citrate (SSC; 1X: 0,15 M NaCl, 0,015 M Na_3-citrate, pH 7,0), 5X Denhardt's solution (Maniatis *et al.*, 1982) 0,5 % SDS and 100 μg mℓ^{-1} denatured herring sperm DNA. All post-hybridization washes were in 2 changes of 2X SSC followed by 2 changes of 0,1X SSC for 15 min each at room temperature. After autoradiography, the membranes were stripped of the probe 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. After pre-hybridization the membranes were re-used and hybridization was as described above.

RESULTS AND DISCUSSION

Hybridization of US isolates to Anaplasma *probes*

Anaplasma-specific DNA probes have recently been isolated from an *A. centrale* genomic library. Probe AC–1 is specific for *A. centrale* while AC–2, AC–3 and AC–4 detect both *A. centrale and A. marginale* DNA (Visser & Ambrosio, 1987).

DNA from the AM-Washington, AM-Florida, AM-Virginia and AM-South Idaho isolates of *A. marginale* was digested with *Eco*R1 and electrophoresed on an 0,8 % agarose gel. After transfer to nylon membranes this DNA was hybridized to labelled probe AC–1, AC–2 and AC–4 respectively.

Probe AC–1 did not hybridize at all to any US-*A. marginale* DNA (Fig. 1). This was expected because of the specificity of AC–1 for *A. centrale* DNA. Probe AC–2 hybridized to different bands on AM-Virginia and AM-Florida (Fig. 2, Lanes 2 and 4). Strong hybridization was obtained to 3 bands on AM-Washington and weak hybridization to another 2 bands from this isolate (Fig. 2, Lane 3). This probe hybridized to 2 bands on

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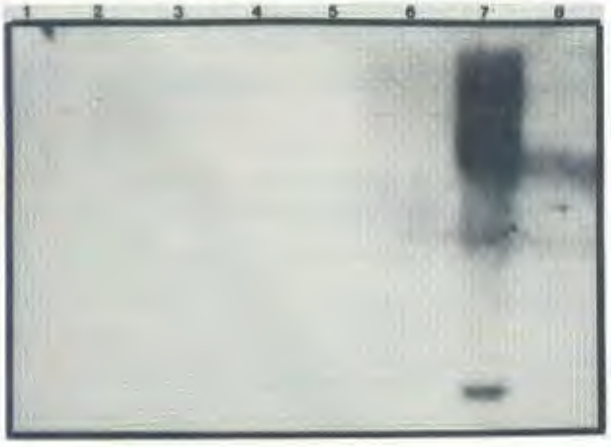

FIG. 1 Hybridization of probe AC–1 to DNA from the US isolates of *A. marginale*. DNA was digested with *Eco*R1, size fractionated on an 0,8 % agarose gel, transferred to nylon and hybridized as described in Materials and Methods.

Lane 1, AM-South Idaho; Lane 2, AM-Virginia; Lane 3, AM-Washington; Lane 4, AM-Florida; Lane 5, Open; Lane 6, *A. marginale*-BW strain; Lane 7, *A. centrale;* Lane 8, Bovine DNA.

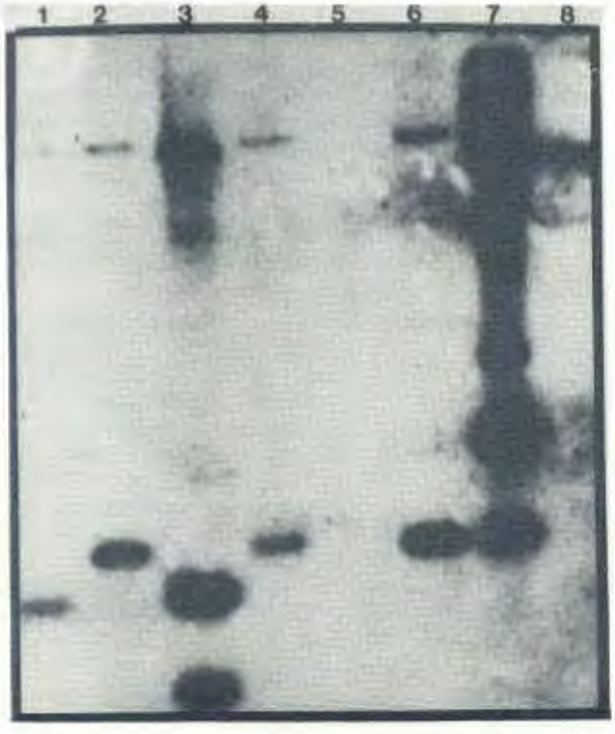

FIG. 2 Hybridization of probe AC–2 to DNA from the US-isolates of *A. marginale*. *Anaplasma* DNA was digested with *Eco*R1. Hybridization and washing conditions were as described in the text.

Lane 1, AM-South Idaho; Lane 2, AM-Virginia; Lane 3, AM-Washington; Lane 4, AM-Florida; Lane 5, Open; Lane 6, *A. marginale* BW-strain; Lane 7, *A. centrale;* Lane 8, Bovine DNA.

AM-South Idaho identical to those on AM-Washington (Fig. 2, Lane 1). However, there was a significant difference in the intensity of hybridization between these two isolates. Identical bands to AM-Virginia and AM-Florida were detected by AC–2 on the BW strain of *A. marginale*. These experiments were repeated with probe AC–4. Because identical hybridization patterns were obtained with AC–2 and AC–4, the blots of AC–4 are not shown.

The presence of similar antigenic determinants on 8 isolates of *A. marginale* has been reported (Palmer, Barbet, Davis & McGuire, 1986). These determinants were present at all stages of acute infection. Morphological differences have been observed among different isolates of *A. marginale*. Some isolates have an appendage which appeared to be lacking in the Florida isolate. Morphological types remain constant between passages in cattle (Ristic, 1980; Carson, Weisiger, Ristic, Thurmon & Nelson, 1974).

Our results suggest that differences, as well as similarities exist on the genome of certain isolates of *A. marginale*. This could support the antigenic and morphological differences as well as similarities between isolates. In addition, the probes originated from an *A. centrale* genomic library, confirming similarities between the DNA of these two species. Similarities of restriction endonuclease digests of *A. centrale* and *A. marginale* DNA have been reported (Ambrosio & Potgieter, 1986).

Hybridization to A. marginale *DNA in the tick vector*

DNA from 20 *A. marginale*-infected *D. andersoni* tick midgut and salivary glands was isolated. After *Eco*R1 digestion this DNA was electrophoresed through a 0,7 % agarose gel and Southern transferred to nylon membranes. Hybridization was with ^{32}P-labelled AC–2 and AC–4 respectively.

FIG. 3 Hybridization of the AC–2 probe to tick mid-gut material. DNA was isolated from *D. andersoni*-infected ticks and hybridized to lug nick-translated AC–2 probe as described in the text. Blots were washed at high stringency (0,1X SSC, 0,5 % SDS, 65 °C for 20 min) and autoradiographed overnight at −70 °C without intensifying screens.

Lanes 1 to 3, Infected *D. andersoni* midgut DNA-*Eco*R1 digested, Lane 4, Infected *D. andersoni* DNA-undigested; Lane 5, *A. marginale* DNA-undigested; Lane 6, *A. centrale* DNA-undigested.

Two strong hybridization bands were obtained with AC–2 (Fig. 3) with midgut-derived DNA. No hybridization was obtained with this probe on salivary gland material or with normal midgut tissue (not shown). The identical hybridization pattern was obtained with the AC–4 probe (not shown).

These results show that AC–2 and AC–4 are able to detect *A. marginale* in tick midgut material. The AC–2

probe detects 8 ng *A. centrale* DNA (Visser & Ambrosio, 1987). The inability of AC–2 to detect *Anaplasma* in the salivary glands could be ascribed to the probe's sensitivity, or to the number of parasites present in the salivary glands. Recently an *A. marginale*-specific probe of 2 kb was isolated and used to detect *A. marginale* DNA in adult *Dermacentor* ticks, infected either as nymphs or adults (Goff, Barbet, Stiller, Palmer, Knowles, Kocan, Gorham & McGuire, 1988). This probe detected the equivalent of a 0,001 % parasitaemia per 5 $\mu\ell$ whole blood. The sensitivity of our probes could be increased about 10-fold by using RNA transcripts (Holmberg, Bjorkman, Franzen, Aslund, Lebbad, Pettersson & Wigzell, 1986). A more sensitive probe would circumvent the problem of detecting low numbers of parasites in the infected tick vector. To date it has only been possible to demonstrate low numbers of colonies of *A. marginale* in the salivary glands of ticks that have been put through a temperature change (Stiller, Kocan, Edward, Ewing, Hair & Barron, 1988). This was done using microscopic techniques.

It was not possible in these experiments to quantitate the amount of *A. marginale* DNA present in the *Dermacentor*-infected material. *Anaplasma* colonies have not been detected in the salivary glands of feeding ticks (Kocan, Wickwire, Ewing, Hair & Barron, 1988). The lack of hybridization of AC–2 to salivary gland material could therefore also be due to the absence of *A. marginale* DNA in this material, and not only to the probe's sensitivity. The possibility that the AC–2 probe could be used to study the developmental stages of *A. marginale* in the tick vector will be investigated.

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