A RECOMBINANT IMMUNODIAGNOSTIC ANTIGEN FOR BOVINE CYSTICERCOSIS

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Abstract. The 70% ammonium sulfate-soluble fraction of the cyst fluid of *Taenia hydatigena* (designated ThFAS) was previously shown to have potential as an immunodiagnostic reagent for bovine cysticercosis. Western blot analysis indicated that the specific reactivity with antibodies in sera of *T. saginata*-infected cattle was associated with a 10 kDa component. Rabbit antiserum to ThFAS identified a homologous antigenic protein from the cestode *Taenia crassiceps*. Consequently, a cDNA expression library was constructed in lambda gt11 using poly A mRNA purified from *T. crassiceps* metacestodes and screened with rabbit antiserum to ThFAS. One strongly reactive clone (designated lambda TCA–2) produced a 123 kDa beta-galactosidase fusion protein which reacted in Western blot with sera from calves experimentally-infected with *T. saginata* and did not react with sera from uninfected calves or from cattle infected with *Fasciola hepatica* or with common gastrointestinal cattle parasites.

INTRODUCTION

Recently, a heterologous antigenic fraction called ThFAS was derived from the sheep cestode, *Taenia hydatigena*, and shown to reliably detect antibodies against *T. saginata* in the enzyme-linked immunosorbent assay (ELISA) (Rhoads *et al*, 1985). ThFAS consisted of a group of high molecular weight proteins (65 to 77 kDa) and a low molecular weight protein of 10 kDa. The 10 kDa protein was subsequently identified as the *T. saginata*-cross-reactive antigenic component of ThFAS (Kamanga-Sollo *et al*, 1987). In this paper, we describe the production by recombinant DNA methodology of an immunodiagnostic epitope of a ThFAS-related 10 kDa protein from *Taenia crassiceps*.

MATERIALS AND METHODS

Parasite extracts. The 70% ammonium sulfate-soluble fraction of the cyst fluid of *Taenia hydatigena* metacestodes (termed ThFAS) was prepared as described previously (Rhoads *et al*, 1985). Antiserum to ThFAS was produced in rabbits (Kamanga-Sollo *et al*, 1987). Metacestodes of *T. crassiceps* were maintained in female Swiss-Webster mice and harvested at 30–day intervals by flushing the opened peritoneal cavity with saline. The metacestodes were washed with saline and extracted for protein by homogenization with 50 mM Tris/HCl buffer, pH 7.5.

Bovine sera. Cattle sera from the following groups of animals were used for Western blot analysis of the recombinant antigen: (1) sera from 10 calves with experimentally-induced *T. saginata* infections (Kamanga-Sollo *et al*, 1987) collected prior to infection, and at necropsy 13 to 26 weeks post-infection; (2) sera from 11 cattle with naturally-acquired infections of *Fasciola hepatica* and (3) sera from 25 cattle from a herd which routinely harbors common gastrointestinal parasites including *Moniezia*, *Ostertagia ostertagi*, *Haemonchus placei*, *Cooperia oncophora*, and *Nematodirus helveitianus*. Sera from these groups were used either individually or as pooled samples.

SDS-PAGE was performed in 1.0 mM-thick vertical mini-slabs using a 6 to 15% acrylamide gradient (Laemmli and Favre, 1973). For Western immunoblots, SDS-PAGE separated proteins were transferred to Immobilon PVDF membranes (Millipore) according to Towbin *et al*, 1979. The membrane was blocked for 1 hour with 0.01 M phosphate buffered saline, pH 7.5 (PBS) containing 5% bovine serum albumin, incubated in test sera (either rabbit or bovine antisera) diluted 1/100 in 1% BSA-PBS for 2 hours, followed by a 1-hour incubation with

either goat anti-rabbit IgG or rabbit anti-bovine IgG peroxidase conjugate diluted 1/1000 in 1% BSA-PBS. The membrane was washed with PBS between incubations. Specific reactions were visualized with hydrogen peroxide and 4-chloro-1-naphthol substrate solution.

Construction and screening of a *T. crassiceps* cDNA library. *T. crassiceps* metacestodes were disrupted by passage through a 20 ga needle. The tissue was separated from the fluid by centrifugation, washed 2X in PBS, and treated with proteinase K:SDS as described (Rishi and McManus, 1988). The nucleic acids were precipitated with ethanol, redissolved in guanidinium isothiocyanate solution and separated into DNA and RNA by cesium trifluoroacetate isopycnic centrifugation (Zarlenga and Gamble, 1987). Poly A mRNA was purified from total RNA as described (Aviv and Leder, 1972). A cDNA expression library was constructed in the Eco R1 site of lambda gt11 bacteriophage DNA using 10 µg of purified mRNA according to the method of Gubler and Hoffman (1983) as modified by Watson and Jackson (1985). The library was screened with rabbit anti-ThFAS as described for Western immunoblots and positive phage lysogenized into the *E. coli* bacteria strain Y1089. Expressed beta-galactosidase fusion proteins from positive clones were analyzed by SDS-PAGE and Western blot with rabbit anti-ThFAS and bovine sera.

RESULTS

T. crassiceps contains a single 10 kDa protein that is recognized by anti-ThFAS serum (Fig 1). Western blot indicates further that the 10 kDa protein from *T. crassiceps* reacts specifically with antibodies in sera from *T. saginata*-infected calves (Fig 2).

Rabbit anti-ThFAS serum identified five strongly positive clones from the *T. crassiceps* cDNA-lambda gt11 recombinant library. The beta-galactosidase fusion protein from one of these clones (designated lambda TCA–2) showed strong reactivity in Western blot with rabbit anti-ThFAS (Fig 3, lane 1), as well as specific reactivity with sera from calves with experimentally-induced cysticercosis infections (Fig 3, lanes 4–6). Lambda TCA–2 did not react with sera from cattle infected with *F. hepatica* or with common gastrointestinal parasites (Fig 3, lanes 2 and 3).

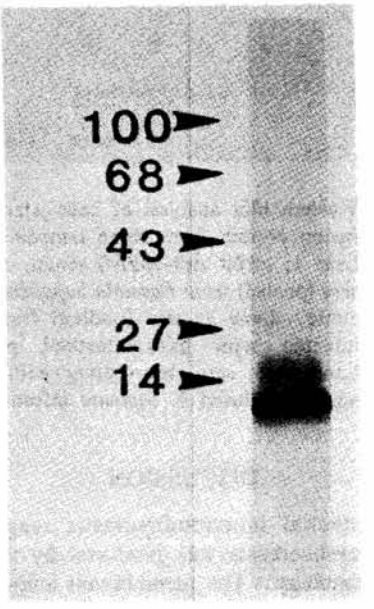

Fig 1 – Western blot analysis of extracted *Taenia crassiceps* proteins with rabbit anti-ThFAS serum.

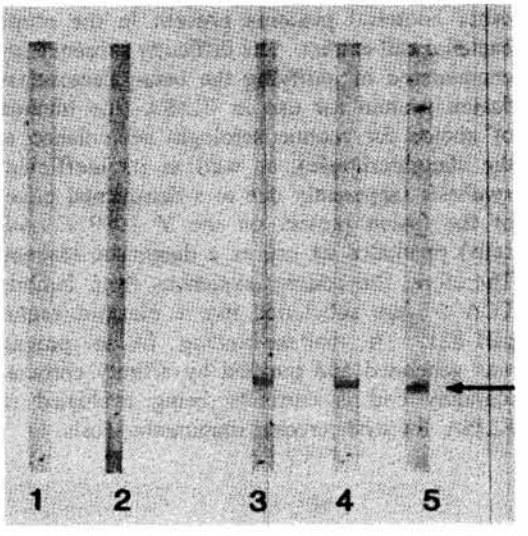

Fig 2 – Western blot of *Taenia crassiceps*. Lanes 1 and 2; sera from uninfected calves. Lanes 3, 4, and 5; sera from calves with experi mentally-induced cysticercosis infections.

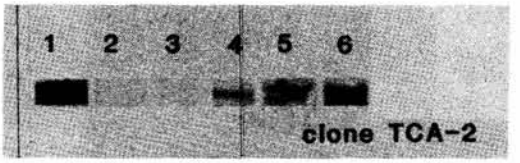

Fig 3 – Western blot analysis of beta-galactosidase fusion protein from clone lambda TCA–2. Lane 1; rabbit anti-ThFAS serum. Lane 2; sera (pooled) from *Fasciola hepatica*-infected cattle. Lane 3; sera (pooled) from cattle infected with gastrointestinal parasites. Lanes 4–6; sera from calves with experimentally-induced *T. saginata* infections.

DISCUSSION

A potential immunodiagnostic reagent for bovine cysticercosis was produced by recombinant technology. The recombinant antigen consisted of a beta-galactosidase fusion protein with an apparent molecular weight of 123 kDa. Although the fusion protein reacted specifically in Western blots with sera from calves with experimentally-induced cysticercosis infections, there was high non-specific reactivity with other bacterial proteins present in the crude bacterial cell extract. The difficulty subsequently encountered in purifying the beta-galactosidase fusion protein for use in ELISA (the method of choice for routine serologic surveillance at the slaughterhouse), as well as its inefficient synthesis (apparently due to a detrimental effect of the fusion protein on the Y 1089 *E. coli* cells) precluded its use as a diagnostic reagent. Therefore, the coding sequences from lambda TCA–2 were subcloned into a plasmid vector pIH 821. A maltose-binding fusion protein was generated and purified by affinity chromatography and is currently being evaluated in ELISA for cysticercosis immunodiagnosis.

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