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Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda)

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ABSTRACT: A humoral immune response of European eel *Anguilla anguilla* to a major antigen in the swimbladder nematode *Anguillicola crassus* was demonstrated by immuno-blotting. Proteins from the nematode were separated using SDS-PAGE (sodium-dodecyl-sulfate polyacrylamide-gel electrophoresis) under reducing conditions and electroblotted onto nitrocellulose membranes, which were incubated in diluted serum from either infected or non-infected eels. Secondary and tertiary antibodies were rabbit anti-eel Ig serum and peroxidase-conjugated swine anti-rabbit Ig antibodies, respectively. Infected eels showed a specific antibody response towards a 43 kDa parasite antigen. The immunoblotting technique seems to provide a serodiagnostic research tool for detecting *A. crassus* infection in the European eel.

INTRODUCTION

The swimbladder nematode *Anguillicola crassus* has been widely distributed in populations of both the Japanese eel (*Anguilla japonica*) and the European eel (*A. anguilla*) in Japan for many years (Egusa 1979), and over the last decade has spread within Europe in natural and cultured populations of *A. anguilla* (De Charleroy et al. 1990, Køie 1991). The endoparasite, whose life cycle includes a tissue migration phase, has the capacity to elicit a host immune response. The present paper reports on the humoral immune reaction of the European eel to this nematode. By using electrophoresis of parasite preparations and immunoblotting with host serum, we demonstrated the production of specific antibodies in naturally infected eels in response to a major immunogenic protein from *Anguillicola crassus*.

MATERIALS AND METHODS

Adult specimens of *Anguillicola crassus* were removed from the swimbladder of naturally infected European eels and sonicated in a sonifier B-12, Branson sonic power (Danbury, Connecticut, USA), at 50 W for 1 min. SDS-PAGE (10 % polyacrylamide) was performed on the parasite suspension (20 µg protein $lane^{-1}$) under reducing conditions according to Laemmli (1970). The separated proteins were electroblotted onto a nitrocellulose membrane (0.45 µm) in a semi-dry blot apparatus (Kem-En-Tec, Copenhagen) (0.4 mA cm^{-2} for 2 h) following the manufacturer's recommendations. Prior to incubation with dilutions of serum from infected and non-infected eels, the nitrocellulose membrane was incubated in blocking buffer (1 % instant non-fat dry milk in phosphate-buffered saline, PBS) for 1 h. Blood, collected by caudal vein puncture from 6 infected and 5 non-infected specimens of *Anguilla anguilla*, was allowed to clot for 1 h, whereupon serum was recovered and frozen at −20 °C until use.

The nitrocellulose membrane with the fractionated parasite proteins was cut into strips and incubated in serum from individual eels (6 infected and 5 non-infected eels, diluted 1:50 in blocking buffer) or blocking buffer alone (control) for 8 h at 4 °C. Detection of bound eel immunoglobulin was performed with 2 additional incubations, first with a rabbit anti-eel Ig serum (diluted 1:1000 in blocking buffer) (K. Buchmann, L. Ø.

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Pedersen & J. Glamann unpubl.) and second with peroxidase-conjugated swine anti-rabbit Ig antibodies (diluted 1:2000) (P 217, DAKO-immunoglobulins A/S, Denmark). These incubations were performed for 1 h at room temperature. Following each incubation the nitrocellulose strips were washed 4 times for 15 min with PBS containing 0.05 % Tween 80. The strips were incubated in 80 mg dioctyl-sulfo-succinate and 24 mg tetramethyl benzidine in 10 ml 96 % ethanol, 30 ml citrate phosphate buffer (pH 5) and 20 µl 30 % hydrogen peroxide, for colour development on immuno-reactive bands.

RESULTS

SDS-PAGE of the sonicated parasite suspension yielded at least 15 distinct protein bands, with molecular weight (MW) ranging from 14 kDa to more than 94 kDa (Fig. 1). The 6 sera from infected eels recognized

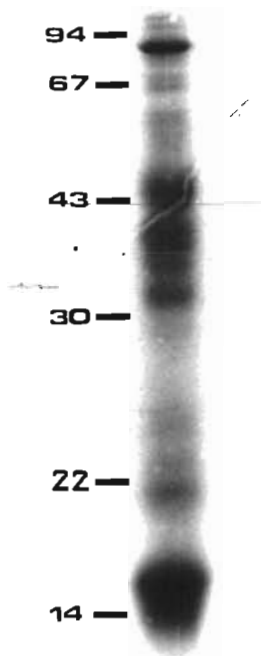

Fig. 1. *Anguillicola crassus*. SDS-PAGE (10 % polyacrylamide) of nematode sonicate run under reducing conditions and stained with Coomassie Blue. Molecular-weight markers are indicated in kDa

specifically at least one of these protein bands, with an MW of ca 43 kDa. In addition, 2 infected eels (Nos. 1 and 2) reacted to 2 proteins of ca 67 kDa. Sera from non-infected eels did not show a reaction to either of these proteins. However, some of both the infected and non-infected eels reacted to 2 proteins with an MW of 60 and 94 kDa, but no reaction was seen in the control strip incubated in secondary and tertiary antibodies only (Fig. 2).

DISCUSSION

This study clearly demonstrates that the European eel is capable of effecting a humoral immune response to an antigen from *Anguillicola crassus*. Similar humoral immune responses of humans to hookworm antigens (Pritchard et al. 1990) and of calves to *Ostertagia* antigens (Canals & Gasbarre 1990) have been reported. Lobos & Weiss (1986) found that a number of antigens in the filarial nematode *Onchocerca volvulus* cross-reacted with serum from humans infected with other parasite species. However, a number of low-molecular-weight antigens (20 to 43 kDa) in *O. volvulus* were found to be non-cross-reacting and more specific, reacting only with serum from onchocerciasis patients. Similarly, we found that serum from some non-infected eels reacted to a few high-molecular-weight antigens from *A. crassus*, whereas only infected eels reacted to the low-molecular-weight antigen (43 kDa).

The immunoblot method provides a serodiagnostic tool which can be used for research purposes to detect *Anguillicola* infections in eel. Due to the relatively high costs of the technique, its usefulness for diagnosis in commercial eel farming is doubtful. However, based on the production of a recombinant non-cross-reacting antigen, a faster and more sensitive serodiagnostic method could be elaborated, corresponding to the work of Lobos et al. (1991) on *Onchocerca volvulus* infections in humans. A number of studies have been conducted on defence mechanisms of fish to helminths (Evans & Gratzek 1989), and an attempt to serodiagnose trematode infections in *Anguilla australis* has been reported (McArthur & Sengupta 1982). However, studies on the isolation and characterization of antigens in fish parasites are sparse. The exact nature of *Anguillicola* antigens should be the subject for future research. Also, a possible protective effect, if any, of the host's humoral immune response to *A. crassus* must be clarified, as we have no indication in the present work that the eel antibody response to this nematode performs such a function.

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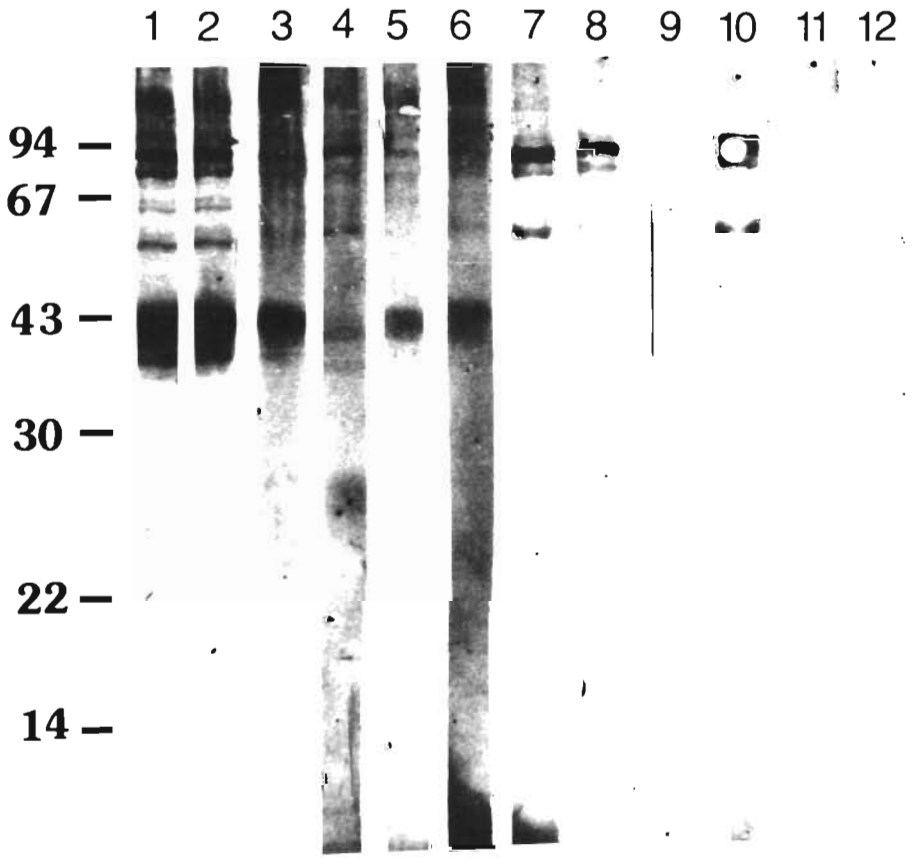

Fig. 2. *Anguilla anguilla* and *Anguillicola crassus*. Immuno-blot showing reaction of serum from infected eels (Lanes 1 to 6) to major parasitic antigens separated with SDS-PAGE as in Fig. 1, and reaction of serum from non-infected eels (Lanes 7 to 11). Lane 12 (control) was only incubated in secondary and tertiary antibody. Molecular-weight markers are indicated in kDa

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