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OBSERVATIONS ON ANTIGENIC ACTIVITY OF *PSEUDODACTYLOGYRUS ANGUILLAE* (MONOGENEA) ON THE EUROPEAN EEL (*ANGUILLA ANGUILLA*)

C. MAZZANTI, G. MONNI AND A.M. COGNETTI VARRIALE
Dipartimento di Patologia Animale, Profilassi ed Igiene degli Alimenti. Viale delle Piagge, 2 Pisa

Introduction

Infections by the gill parasitic monogeneans, *Pseudodactylogyrus sp.* are frequent in commercial eel farming in Italy. These platyhelminths were introduced from the Pacific area and first reported from Europe by Golovin (1977), Molnár (1983) and Lambert *et al.* (1984). The genus *Pseudodactylogyrus* is now widespread in populations of *Anguilla anguilla* in European natural waters (Køie, 1991).

Information on the immune response of teleosts against metazoan parasites is limited. It has been suggested that immunological responses, both cellular and humoral, are important in regulating the parasite burden of fish and in influencing changes in parasite population (Thomas & Woo, 1995). Fish immune response has been studied against monogenean parasites such as *Pseudodactylogyrus bini* (Buchmann, 1993) and *Diplectanum aequans* (Cecchini *et al.*, 1996). In the present study we examined the humoral immune response of European eel to the gill parasite *Pseudodactylogyrus anguillae*.

Materials and methods

Fish and blood samples:

Blood samples were drawn by caudal vein puncture from 10 infected eels, reared in a closed-circuit, intensive breeding farm in Castelnuovo Val di Cecina (Tuscany). Control blood samples were drawn from 5 non-infected eels from the Orbetello Lagoon (Tuscany). After collecting, the blood was allowed to clot for 1 h at room temperature and overnight at 4°C. Next day, samples were first centrifuged at 2500 g for 10 min and then at 2000 g for 10 min. The sera were stored at -20°C.

Parasite Collection and Treatment:

Adults of the gill parasite *Pseudodactylogyrus anguillae,* were taken from eels reared in the closed-circuit, intensive breeding farm in Castelnuovo Val di Cecina (Tuscany). After collection, parasites were stored in Ringer solution at -20°C. Then parasites were sonicated with three cycles of 30 s on and 30 s off (sonifier Vibracell, Sonics & Materials Inc.). Whole homogenate was used as immunogen.

Electrophoresis & Western blotting analysis:

Proteins from sonicated parasites were separated by SDS-PAGE according to the method of Laemmli (1970) using a Mini Protean II dual slab gel system (Bio-Rad). The samples were mixed with an equal volume of reducing buffer and heated at 70°C for 10 min to denature the proteins. Molecular weight standard (Low Range Bio-Rad Cat. No. 161-0304) and samples were loaded onto the stacking gel and electrophoresed into a 12 % polyacrylammide gel.

Eel sera were tested for reaction to antigens of *Pseudactylogyrus anguillae* using Western Blot analysis (Towbin *et al.*, 1979). Proteins from sonicated parasites separated by SDS-PAGE were electroblotted onto nitrocellulose paper. The nitrocellulose membrane was cut into strips and incubated in serum from individual eels (10 infected and 5 non-infected eels, diluted 1:50 in blocking buffer) or blocking buffer alone (control) for 2 h at room temperature. The chemical bond between antigens of *P. anguillae* and eel immunoglobulins was detected with two additional incubations, first with a rabbit anti-eel IgM serum (Monni *et al.*, unpublished), diluted 1:1000 in blocking buffer for 2 hours and second with goat anti rabbit IgG

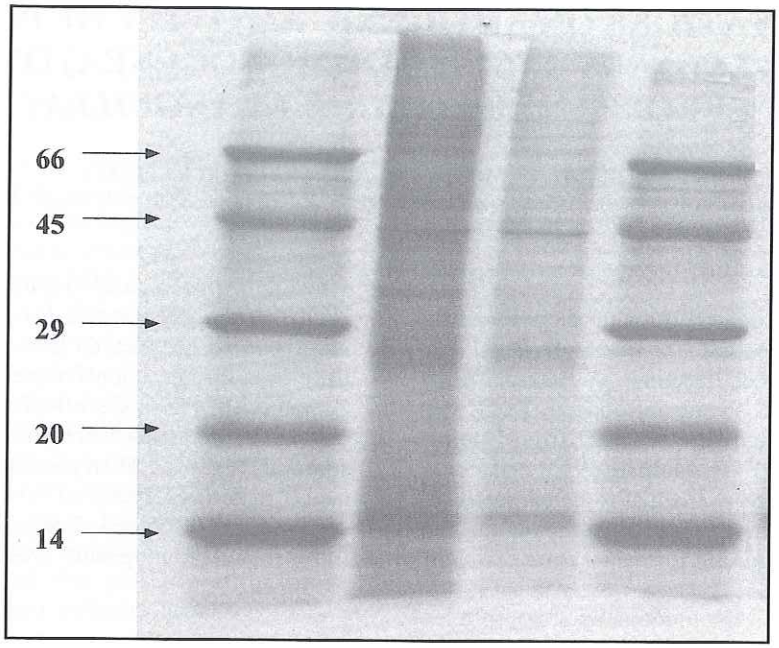

Figure 1 Coomassie blue stained 12% SDS-PAGE slab of a sonicated *Pseudodactyylogyrus anguillae* preparation run under reducing conditions. Molecular weight markers in kDa are indicated.

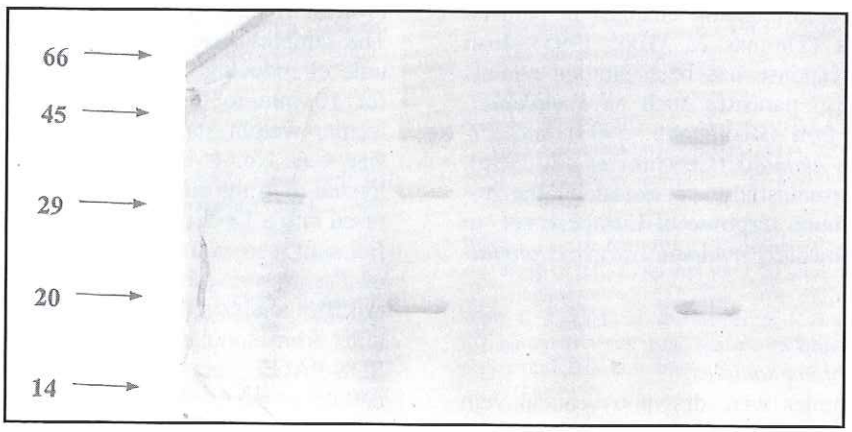

Figure 2. Western blotting of *Pseudodactylogyrus anguillae* antigens incubated in serum from naturally infected eel. Molecular weight markers in kDa are indicated. Antigens are in lanes 1 and 5, Moleular weight markers are in lanes 3 and 7

peroxidase-conjugated (Sigma) diluted 1:2000 in blocking buffer for 1 h. The strips developed immuno-reactive bands in a solution made of 30 mg of 4-Chloro-1-naphtol (Fluka) in 10 ml of methyl alcohol, 50 ml of deionised water and 30 µl 30% hydrogen peroxide.

Results

SDS-PAGE of the sonicated parasites suspension yielded at least 35 distinct protein

bands, with molecular weight (MW) ranging from 14 kDa to more than 66 kDa (Fig.1).
The 10 sera from infected eels recognised specifically (in Western Blot analysis) at least three of these protein bands with a MW of 14, 29 and 31 kDa (Fig.2). One of these sera recognised five protein bands of 14,19,20,29,45 kDa.
Sera from non-infected eels did not show a reaction to either of these proteins of low molecular weight. However, some of these non-infected eels reacted to proteins of a high molecular weight (between 66 and 97 kDa).
No reaction was seen in the control strip incubated in secondary and tertiary antibodies only.

Discussion

Our research shows clearly that European eel has specific immune response against *Pseudodactylogyrus anguillae*.
A specific immune response has been reported also against *P. bini* in Buchmann (1993) where individual eel sera from infected eels reacted weakly with two parasite antigens (with MW of 60 kDa and >94kd) while non infected eels did not respond to these antigens.
On the contrary eels infected by *P. anguillae* show a reaction to protein bands of low MW (14,29 and 31 kDa) while some non infected eels reacted to proteins of a high MW as it is reported also in Buchmann (1991) where humoral immune response against the swimbladder nematode *Anguillicola crassus* has been clearly described.
Observations on cultured eels have shown that *P. anguillae* moves actively on the gill surface, whereas *P. bini* is more sedentary (Køie, 1991). The large hamuli of P. anguillae may cause haemorrhage and damage to the tissue (Buchmann et al., 1987a, b) while *P. bini* with small hamuli is often surrounded by an extensive tissue reaction.
SDS PAGE electrophoresis of the helminth preparation of *P. anguillae* shows a greater number of protein bands (35) in comparison with those reported in *P. bini* (18 bands): this could be explained with a more complex structure of *P. anguillae* and the following wide reaction of the immune system, observed in this work, of eels infected by this parasite could reflect the greater damage of the gill tissue by *P. anguillae*.

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