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Aquat. Living Resour., 1988, **1**, 67-69

Serological analysis of *Bonamia* in *Ostrea edulis* and *Tiostrea lutaria* using polyclonal and monoclonal antibodies

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Accepted November 20, 1987.

Mialhe E., V. Boulo, R. Elston, B. Hill, M. Hine, J. Montes, P. Van Banning, H. Grizel, *Aquat. Living Resour.*, 1988, **1**, 67-69.

Abstract

Murine monoclonal and polyclonal antibodies to antigens of *Bonamia ostreae* were assessed for serological comparison of parasites associated with different geographical origins of flat oysters, *Ostrea edulis*, and with *Tiostrea lutaria*. All *Bonamia* isolates from *O. edulis* reacted similary with polyclonal and monoclonal antibodies. The parasite associated with *Tiostrea lutaria* which lacks reactivity with 20 B2 MAB must be considered as a serologically distinct form of *B. ostreae*.

Keywords : *Bonamia, Ostrea edulis, Tiostrea lutaria*, polyclonal and monoclonal antibodies, serological comparison.

Comparaisons sérologiques de Bonamia parasite d'*Ostrea edulis* et de *Tiostrea lutaria* à l'aide d'anticorps polyclonaux et monoclonaux.

Résumé

Des anticorps polyclonaux et monoclonaux de souris reconnaissant des antigènes de *Bonamia ostreae* ont été utilisés pour réaliser une comparaison sérologique entre les parasites du genre *Bonamia* de l'huître plate, *Ostrea edulis*, de différentes origines et de *Tiostrea lutaria*. Tous les *Bonamia* purifiés à partir des tissus d'*O. edulis* réagissent de la même manière avec les anticorps polyclonaux et monoclonaux. Le parasite de *T. lutaria* qui ne réagit pas avec l'anticorps monoclonal, 20 B2, est considéré comme une souche sérologique distincte de *B. ostreae*.

Mots-clés : *Bonamia, Ostrea edulis, Tiostrea lutaria*, anticorps polyclonaux et monoclonaux, comparaison sérologique.

Following substantial mortalities of the European flat oyster *Ostrea edulis* in France in 1979, a new protozoan parasite was described as *Bonamia ostreae* (Pichot *et al.*, 1980). The hemocytic disease caused by this pathogen, bonamiasis, spread rapidly to almost all of the oyster farming areas of Brittany and to other European countries including England, Spain, the Netherlands and Ireland.

Recently, a similar disease was observed in several populations of *O. edulis* from the west coast of North America (Elston *et al.*, 1986). On the basis of ultrastructural characteristics, host specificity and transmissibility, the responsible agent was identified as *B. ostreae.* Moreover, examination of historical records lead to the hypothesis that the microcell disease exported in California in the 1960's was identical with bonamiasis and that the disease spread from North America to Europe.

Bonamiasis was also recently detected in the New Zealand dredge oyster *Tiostrea lutaria* (Hine, pers. comm.). Structural similarities exist between the New Zealand parasite and *B. ostreae* which suggest near conspecificity, although small differences in size and the structure of some organelles are apparent. Thus, bonamiasis, may affect several oysters species and several geographic areas.

From epidemiologic and taxonomic points of view, it is important to define the relationships between the causative agents of the multiple reports of bonamiasis. Following the example of studies developed in human and veterinary pathology to characterize protozoan parasites, serological analysis will permit better definition of the relationship of different parasite strains (Bhatia *et al.*, 1987; Grimaldi *et al.*, 1987; Krieger *et al.*, 1985).

Specific polyclonal (PAB) and monoclonal (MAB) antibodies have been prepared against French *B. ostreae* (Rogier *et al.*, in prep.). These reagents constitute the panel of tools used to develop the investigations reported here. In the present study, we incorporated these antibodies into an indirect immunofluorescence technique to compare the *Bonamia* organisms infecting *O. edulis* and *Tiostrea lutaria* from widespread areas around the world.

MATERIALS AND METHODS

Bonamia parasites

Infected oysters were collected in Quiberon Bay (France), England, Galicia (Spain), Yerseke Bank (Netherlands), Cork (Ireland), Washington state (USA) and Faveaux Strait (New-Zealand). Impression smears of heart tissues were air-dried and fixed by immersion in acetone (10 minutes).

Antibodies

- *Bonamia ostreae* — specific mouse immunserum (LPGIM n° 22) purified with acetone extracted healthy oyster tissue powder (w/w).
- Control mouse serum (LPGIM n° 36).
- *Bonamia ostreae* specific hybridomas were grown in pristane-pretreated mice. Six monoclonal antibodies, 20 B2, 15 C2, 10 G9, 16 G5, 2 E3, 16 F11 were purified from ascite-fluids by affinity chromatography on immobilized protein A.
- Control mice monoclonal antibodies D7 E7, 8 A7 and M4 A3, specific to human antigens, were provided by F. Paolucci (Sanofi Recherches, Montpellier).

Indirect immunofluorescence

The slides were overlayed with polyclonal (PAB) or monoclonal (MAB) antibodies, specific or control, diluted in PBS (Phosphate 10 mM, NaCl 150 mM, pH 7.4).

The immunsera were diluted 1/100 and the purified monoclonal antibodies were used at a final concentration of 100 µg/cm^3. After a 30 minutes incubation period at ambiant temperature in a moist chamber, the slides were washed with PBS and then overlayed with FITC-conjugated goat antiserum to mouse immunoglobulins (Diagnostic Pasteur), diluted in the same buffer containing 0.01% Evans-Blue.

The slides were again incubated as described and washed in PBS. A cover slip was then placed by using glycerin-buffer mounting fluid. The slides were examined for bright green fluorescent cells that were morphologically consistent with *Bonamia.* The control antigens consisted of heart smears prepared from healthy oysters.

RESULTS

All *Bonamia* isolates reacted with polyclonal antibodies from the LPGIM n° 22 immunserum, specific

Table 1. — Reactions of *Bonamia* isolates with a panel of six monoclonal antibodies.

Monoclonal Antibodies	15 C2	20 B2	2 E3	10 G9	16 G5	16 F11
France	+	+	+	+	+	+
England	+	+	+	+	+	+
Spain	+	+	+	+	+	+
Holland	+	+	+	+	+	+
Ireland	+	+	+	+	+	+
U.S.A.	+	+	+	+	+	+
New Zealand	+	−				

for *Bonamia ostreae* purified from French oysters. However, the fluorescence intensity was less marked with *Bonamia* from infected *Tiostrea lutaria* than with *Bonamia* from infected *Ostrea edulis* oysters.

The reactions among the different geographic isolates of *Bonamia* with individual monoclonal antibodies are listed in *table* 1. The 15 C2 MAB, whose the fluorescent pattern in linked to the parasites membrane, reacted with all *Bonamia* isolates, whereas the 20 B2 MAB, which also reacts with the membrane, distinguishes distinctly between *Bonamia* from *Ostrea edulis* and *Bonamia* from *Tiostrea lutaria.*

The other monoclonal antibodies, whose corresponding epitopes are cytoplasmic, reacted similarly with European and American *Bonamia* from *O. edulis.* Insufficient heart smears of *T. lutaria* were available to perform the I.I.F. assay with these specific MAB. All the control assays with polyclonal and monoclonal antibodies were negative for fluorescent reactivity.

DISCUSSION

Until now the methods of morphological pathology have been used to identify parasites of molluscs. Such techniques, however, are inadequate for epidemiologic and taxonomic studies since they lack sufficent specificity. Serological investigations with PAB and MAB developed in this study represent an original and useful approach in molluscs for parasite characterization. A primary objective of the present study was to compare antigenic markers from *Bonamia* occurring in widely separated geographic areas and from different host species.

No antigenics differences were observed between *Bonamia* from the U.S.A. and European countries. Thus, this work substantiates the hypothesis of Elston *et al.* (1986) that *Bonamia ostreae* was introduced from Elkhorn Slough to France and to Washington state. Following its introduction into France the disease spread rapidly through European countries where it now exists as a single serotype.

The lack of reactivity of the 20 B2 MAB with *Bonamia* associated with *T. lutaria* is significant from a taxonomic point of view. This MAB recognizes a prominant epitope on the cytoplasmic membrance of *B. ostreae.* Thus, the parasite of *T. lutaria* with lacks this epitope must be considered serologically distinct from *Bonamia ostreae* with which it shares another major membrane epitope. Thus, both structural and antigenic differences between the parasites in the two host species indicate that the *T. lutaria* parasite should be considered as a different species than *B. ostreae.*

These results obtained with polyclonal and monoclonal antibodies represent new methods of investigation in the characterization of pathogens of molluscs. Other molecular and biochemical techniques like protein electrophoresis or analysis of genomic content by restriction endonucleases or DNA cloning should also be developed. These methodologies can now be realistically attempted as the result of purification protocols recently developed for several molluscan pathogens (Mialhe *et al.*, in press; Mialhe *et al.*, 1985).

Acknowledgements

The authors wish to thank D. Hugh-Jones, Atlantic Shellfish ltd. Cork, Ireland for his help.

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