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Prophylactic strategies and zootechnic measures, recent advances

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Abstract — *The spread of major diseases of molluscs around the world and the increase of transfers need the development of adapted prophylactic strategies and the increase of research in epidemiology, immunology and genetics.*

The prophylactic strategies must be based on common laws with the establishment of a list of declarable pathogens, with basic sampling rules (sample size, frequency, definition of geographical areas) and with reglementation of the internal transfers. Moreover, specific adapted diagnosis should be developed to increase the sanitary control performance (e.g. ELISA test, DNA probe, cell culture).

Concerning the epidemiology, specific experimental plans should be realised, when abnormal mortalities occur in a growing area. In the case of an infectious disease the epidemiology studies should be concerned, the relationship beetween the culture techniques and the disease, the repartition of the disease, in terms of prevalence and infection ratios, experimental infection in the field and in the lab, the possible effects of the environmental components.

On the other hand, specific zootechnical research should be increased, particulary in genetics which is the main way to find applicable solutions to control the diseases. Selection of resistant strains, hybridization, gene transfer should permit the formation of new adapted strains. Finally, with the development of new models in mollusc pathology, like ***Bonamia*** *and Rickettsies and hemocytes, the study on defense mecanism, humoral and cellular response, enter in a new approach which should permit to understand better this mechanism and which should permit to select characters of resistance.*

INTRODUCTION

Over the past twenty years the development of hatcheries and the case of transportation facilities have increased the exchanges of alive animals between countries. Molluscs have followed the same way and the commercial exchanges concern the different age populations. In parallel, five recensed epizooties have occured in the world and some of them have spread in different countries. The best example concerns the parasite

Bonamia ostreae, native to the U.S.A., which was introduced to France with the importation of juveniles and extented to Spain, Netherlands, England and Ireland (Elston et *al.*, 1986; Mialhe et *al.*, 1988; Grizel, 1985).

In front of this situation, the laws appear completely inadapted, and the number of efficient applied actions to reduce the disease is very low. Also, it has become very important to develop new research in view of enhancing the zoosanitary control and to perform zootechnic prophylaxies.

ZOOSANITARY CONTROL

The zoosanitary control is based on laws which are very variable between countries. Moreover, none of them took into account the notion of pathogen. Now, with progress in the knowledges of pathogens, it is possible to establish a list of these. The interest of this list is considerable because it should permit to :

1. develop specific diagnosis methods, easier to use than histological techniques.
2. propose adapted sampling plans in definite geographical area in order to reinforce the quality of the control and to regularly obtain a map of the epidemic situation.
3. define correctly, disease by disease, the control rules (frequency, admissible prevalence levels) and the management rules for each disease (transfer, definition of free areas, contaminated areas and so on).

RECENT ADVANCES IN DIAGNOSIS METHODS

One of the characteristic in mollusc pathology is the absence of cell cultures used in Vertebrates for the diagnosis of some microorganisms. The common method remains the histological technique which is useful to observe different pathogens. But, on the other hand histology takes a lot of time, does not permit quantification and it is an expensive and non sensitive technique. These reasons have conducted Boulo et *al.* (1989) to perform a serological diagnosis based on the E.L.I.S.A. method to detect *B. ostreae*. This progress proceeds from the obtention of purified parasite (Mialhe et *al.*, 1985, 1988) which have opened important research ways in mollusc pathology. So, the main results concerning the diagnosis comparison between smears and E.L.I.S.A shows for an equal sensitivity a saving of time of around six hours and a cheaper cost (Boulo, 1989). In the case of low prevalence of *B. ostreae* and for an equal time control effort, the use of E.L.I.S.A. diagnosis kit gives a better response because the control can be easily made on a large sample.

Moreover, before choosing the E.L.I.S.A method, the I.I.F. technique with polyclonal and monoclonal antibodies has been tested. This method is more sensitive than the smears technique but compared to E.L.I.S.A it requires microscopic observation. In fact, I.I.F. stays very useful and efficient in the case of spat control for which the quantity of hemolymph ponctioned is not sufficient. Serological diagnosis, according to different

methods (sero agglutination, I.I.F., E.L.I.S.A.), used to depend on the host and on the parasite group (virus, bacterias, protozoan, etc...) and localization (internal or external). It could be also interesting in the near future to develop other new techniques, such as DNA probe and in every case to reinforce the research on the obtention of cell lines. The absence of this tool reduces considerably the potential of actions in mollusc pathology.

PROGRESS IN LAWS : HARMONIZATION

Different international organizations : Office International des Epizooties (O.I.E.), Conseil International pour l'Exploration de la Mer (C.I.E.M.) try to push the different responsibles and actors of the exchanges of animals to take into account the major risks presented by the diseases in aquaculture. The role of scientists in this action is very important to sensibilize the administration, inform and educate the farmers and perform diagnosis methods and sampling plans in each geographical area. Many countries have no laws and none of them has made a list of declarable and undesirable diseases. Nevertheless, some pathogens are now well known, like *Bonamia ostreae, Minchinia nelsoni, M. costalis, Marteilia refringens, Perkinsus marinus* and it could be possible to define, disease by disease, importation and transfer rules.

The putting together of ideas by the different organizations should be beneficial to an adapted aquaculture regulation. Compared to the zoosanitary regulation in force for vertebrates, the result is exactly the same. The only question is : does the country prefer to develop aquaculture or commercial exchanges ?

In the second case the philosophy becomes completely different and does not need a so strong regulation, but one must not forget the possible impact of a disease on wild stock.

ZOOTECHNIQUE MEASURES

Different zootechnic measures should be envisaged when an infectious disease appears. Some of them result in the acquisition of knowledge on parasites, mainly the cycle development, the infectious period and the relationships between the pathogen and the farming techniques (density, growth methods) and beetween the pathogen and the environment (t°, s ‰; O_2). Each disease is particular but general and adapted experimental plans can be used when abnormal mortalities occur in a geographical area.

Presently, the number of applicable zootechnic measures remains very low, due to the characteristics of the shellfish culture conducted in open sea and also to the farmers who do not apply unanimously the recommendations.

Also, in front of this different difficulty, it seems better to work on the host using the different techniques of genetics. Compared to the results obtained on insects and on plants, few works and results have been obtained on molluscs. The main data concern the formation of a strain

with increased resistance to *Minchinia nelsoni* (Haskin et Ford, 1979; Ford et *al.*, 1988). This selected strain came from broodstock picked up on oysters bed exposed naturally to the MSX disease. The successive breedings between oysters of this strain show an increase in survival ratio.

In spite of the results, these experiments remain critizable because it is not possible to analyse the genetic and other components, like veracity of the control, evolution of the infectivity of the parasite.

Still, this strategy can be used with some modifications in the concept.

The other interesting ways to exploit concern individual resistance, polyploïdization and gene transfer.

Individual resistance against a disease should be exploited using breeders which have survived inoculation by a parasite. Actually any results concerning molluscs have been reported, but after the reproduction of bonamiasis using purified pathogen (Bigot-Vuillemin, 1987) a monitored broodstock is in creation. It will be useful to examine the heritability of the resistant character.

Different techniques of polyploïdization have been applied successfully on different species, *Crassostrea virginica, C. gigas, Ostrea edulis* and *Ruditapes philippinarum* (Allen, 1986; Gendreau, 1988; Dufy, 1988).

As for fish, this experiment has been realized to increase the growth and the quality of the meat. However, in one case the polyploïdization of hybrids *Salmo trutta x Salmo salar* have given resistant individuals (Dorson et Chevassus, 1985).

It appears interesting to test hypothesis leading to an existing identical phenomenon with molluscs.

Finally, the research on gene transfer should be also very interesting. The advance of the concept and of the results on plants and insects requires attention, particularly the different models used to fight against some Virosis (Cuozzo et *al.*, 1988; Hemenway et *al.*, 1988).

An effort in this research should surely be beneficial and should permit to find applied solutions to resolve the disease problem.

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