PATHOGENS IN AQUACULTURE : SPECIFIC PATHOGENS OR HUMAN CONTAMINATION RISKS

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Introduction

The vastness of aquaculture ventures worldwide has taken impressive leaps during the past three decades, and a large portion of this pertains to mariculture. Significant strides have been made in the design and implementation of more efficient and cost effective growing areas, feed, and harvesting techniques. Moreover the economic impact of aquaculture will certainly increase in the years ahead.

As the development of mariculture has advanced, it has become increasingly apparent that one essentially unsolved problem is the prevention of mass mortalities and cure of mass morbidities among species of aquaculture interest. Indeed, epizootic in commercially exploited species have repeatedly struck the related industries, sometimes causing their virtual extinction (1).

However, the development of aquaculture products consumption associated with the development of mariculture can lead to an other acute problem : the human contamination risks. Indeed, due to the rearing locations, the commercially exploited species are capable of accumulating, from the surrounding water, large numbers of microorganisms. Thus, without themselves contracting a disease, the cultivated species can act as passive carriers of microorganisms pathogen to man (2-4).

Moreover descriptions of microorganisms are often performed among species of aquaculture interest, but the range of infectivity of these agents in the host species is not always determinated and, in particular, it is not known whether any of them are able to produce disease in homeothermic animals including man (5). Indeed, when a microorganism is detected in a species of aquaculture interest, it is often difficult to determine if the agent is specific of the host or if the animal is only a carrier.

For this presentation, because of the great number of species of aquaculture interest, it was necessary to make a choice. Thus, we will only present an overview of microorganisms observed among the marine bivalve molluscs. Indeed, among cultivated marine species, bivalves constitute a group of major economic importance. Moreover, due to their efficient filter-feeding mechanism, bivalve molluscs are capable of accumulating from surrounding sea waters, large numbers of specific or non-specific microorganisms.

Virus families	*Bivalve host*
Iridoviridae	*Crassostrea angulata - Crassostrea gigas*
Papovaviridae	*Crassostrea virginica - Crassostrea gigas - Crassostrea commercialis - Ostrea edulis - Ostrea lurida - Mya arenaria*
Herpesviridae	*Crassostrea virginica - Crassostrea gigas - Ostrea edulis - Mercenaria mercenaria*
Togaviridae	*Ostrea lurida*
Retroviridae	*Mya arenaria*
Paramyxoviridae	*Mya arenaria*
Reoviridae	*Crassostrea gigas - Crassostrea virginica - Ostrea edulis - Tellina tenuis - Mercenaria mercenaria*
Picornaviridae	*Mytilus edulis*

Table 1. Occurrence of viruses in marine bivalves

Agents : virales

Farley (6) has summarized the informations pertaining to viruses and virus-like lesions in marine molluscs and has attempted to categorize them systematically into appropriate families. From their morphology and development, Virales occurring in bivalves may be grouped with the Iridoviridae, Papovaviridae, Herpetoviridae, Togaviridae, Retroviridae, Paramyxoviridae, Reoviridae and Picornaviridae (Table 1).

Some virus types are specific of invertebrates like Iridiviridae. For example, "la Maladie des branchies" ("gill disease") has been identified as the cause of recurrent mass mortalities of Portuguese oysters *Crassostrea angulata* in France since 1966 and led to the quasi disappearance of this species in the late 1970's. Electron microscopy demonstrated that the large globular cells, which become apparent during the necrotic changes of the affected gills are hypertrophied cells containing viral entities. The various properties, as well as its assemblage within the cytoplasm, characterize the entity as a member of the Iridoviridae (7).

For other virus types, it is sometimes more difficult to determine the exact host specificity of these agents. Indeed, some molluscan viruses are morphologically similar to oncogenic viruses found in homeothermic animals. Appeldoorn and Oprandy (8) have demonstrated a causal relationship between the occurrence of haematopoietic neoplasia in *Mya arenaria* and the presence of a virus ressembling B type retroviruses. The range of infectivity of this type of viruses has not yet been determinated and, in particular, it is not known whether any of them are able to induce disease in homeothermic animals including man.

Considerable impact, on the other hand, results from the capacity of bivalves to accumulate human viruses from contaminated waters (Table 2). Numerous studies have shown that molluscs can serve as virus carriers and can accumulate high levels of particles (9-11). Survival of viral particles may be higher in oysters tissues than in sea water (9). Thus, there are several reports on outbreaks of infectious hepatitis epidemics traceable to the consomption of shellfish.

Agents : bacteria

Due to their efficient filter-feeding mechanism, bivalves are capable to accumulate from surrounding sea waters, large numbers of micro-oganisms, and consequently harbour an exceptionally rich bacterial flora, including a majority of Gram-negative species (*Achromobacter, Aeromonas, Flavobacterium, Pseudomonas* and *Vibrio*), and Gram-positive representatives of *Bacillus, Corynebacterium* and *Micrococcus*. The role of bacteria as organisms causing disease in pelecypods is not always clear. Species of *Vibrio* and *Pseudomonas* are normal and frequently the dominant constituents of the natural bacterial flora of the digestive tract of clams (12). Indeed, in nature, free bacteria bivalves do not exist. However, vibrios, pseudomonads and other Gram-negative forms are frequently associated with mortalities of adult bivalves and implicated in most bacterial diseases affecting this invertebrate group (13-14). Thus, the distinction between non-pathogenic species and true pathogens is often difficult, as is the analysis of factors determining the apparent switching of a bacterium from non-pathogenicity to pathogenicity. Moreover, there appears to be a quantitative aspect in the host-microorganism relationship. In considering the equivocal reports on the effects of bacteria on marine molluscs, we must also evaluate these microorganisms in terms of the "infection versus disease" concept. Hence, normally bacterial *infections* exist in bivalves without causing bacterial diseases.

By an other hand, without themselves contracting a bacterial disease, some bivalves can act as passive carriers of bacteria pathogenic to man. For example, the halophilic agent *Vibrio parahaemolyticus* is worldwide distributed in oceanic and coastal waters and has repeatedly been isolated from lamellibranch. This microorganism is the major source for bacterial shellfish poisoning in various parts of the world, particularly in Japan (15). However, the same agent has been incriminated directly of causing disease and mortalities in marine bivalves. Thus, Liposky and Chew (16) classified the organism as "a suspected marine molluscan pathogenic bacteria". However, some techniques, including DNA homology, have indicated that some earlier identifications of bacteria from diseased molluscs as *Vibrio parahaemolyticus* may be in doubt (17).

Agents : rickettsiae, chlamydiae and mycoplasmas

In the past few years, evidence accumulates that rickettsiae, chlamydiae and mycoplasmas can live or survive in marine bivalves (Table 3). Some appear to be capable of causing disease and possible death in these molluscs. All three groups contain forms that produce severe and sometimes fatal diseases in man. Whether forms observed in bivalves originate from contaminated water, is at present unknown. Because they are potentially pathogenic to man, rickettsiae, chlamydiae and mycoplasmas occurring in commercially exploited marine bivalves deserve special attention.

Rickettsiae have been detected in several commercially important marine bivalve species (*Crassotrea gigas, Crassostrea virginica, Ostrea edulis, Mytilus edulis*, etc). Aside from the distinct pathological changes observed in individual infected host cells, the overall pathology caused by most of these agents appears to be light, since usually only a few cells are affected. Although Comps *et al.* (18) found rickettsiae only in diseased *Crassostrea gigas*, they were unable to identify these microorganisms as primary cause of death of the oysters. In contrast, for example, the presence of rickettsiae in *Pecten maximus* (19) was definitely to be associated with disease. The cycle and mode of transmission of bivalve-infecting rickettsiae are unknown. Direct passage from host to host appears probable. Rickettsiae pathogenic to man require passage through an arthropod host. As an exception, *Coxiella burnetii*, the etiological agent of Q fever, is unusually stable outside host cells and is transmitted to man by infected dust or droplets. Buchanan (20) and Comps (21) believe that the rickettsiae present in *Crassostrea gigas* and *Tellina tenuis* may be related to *Coxiella*. Buchanan (20) was able to cultivate the *Tellina tenuis* agent in chick embryo yolk sac at 37°C, which indicates that its life cycle possibly involves a warm-blooded host. Buchanan concluded that the microorganism might have an alternate host in shore birds. In the light of these findings and because it is recognized that several zoonoses including coxiellosis (22) can be transmitted from birds to humans, it has become important to establish the true nature of the rickettsiae present in marine bivalves. Even if the molluscan rickettsiae would lack the capacity to infect humans, some detriment must be expected to result from ingestion of heavily infected molluscs. These microorganisms are known to produce strong toxins capable of killing small mammals within a few hours (23).

Chlamydiae have been detected in several molluscs species (*Mercenaria merenaria, Crassostrea angulata, Mytilus edulis*, etc...). As well the rickettsiae, the mode of transmission of bivale-infecting chlamydiae, as well their possible pathogenicity to humans, are unknown.

Some mycoplasmas are detected among bivalves, but no futher information is available on the host-parasite relationship and specific identities of these microorganisms.

Whether the ubiquity and apparent low pathogenicity of these obligate prokaryotes to marine bivalves would indicate a long standing of the association, remains to be established. The possibility exists that the molluscs merely serve as tolerant carriers

Virus families	Genera	Diseases
Picornaviridae	Enterovirus	Poliomyelitis - Paralysis - Myocarditis - Respiratory infection - Meningitis
	Hepatovirus	Infectious hepatitis
Reoviridae	Réovirus	?
	Rotavirus	Gastro-enteritis
Caliciviridae	Calicivirus	Gastro-enteritis
Coronaviridae	Coronavirus	Gastro-enteritis
Adenoviridae	Adenovirus	Gastro-enteritis

Table 2. Viruses pathogenic to man observed in water

Microorganisms	Bivalve host
Rickettsiae	*Crassostrea virginica - Crassostrea gigas - Ostrea edulis - Mytilus edulis - Mytilus californianus - Mercenaria mercnaria - Mya arenaria - Tellina tenuis - Pecten maximus - Tapes japonica - Patinopecten yessoensis*
Chlamydiae	*Crassostrea gigas - Crassastrea angulata - Mytilus edulis - Mytilus galloprovincialis - Mercenaria mercenaria - Ruditapes decussatus- Argopecten irradians*
Mycoplasmas	*Crassostrea virginica - Tellina tenuis*

Table 3. Occurrence of Rickettsiae, Chlamydiae and Mycoplasmas in marine bivalves

for these organisms, which inflict overt pathogenicity on other (vertebrate?) hosts.

Conclusions

Among the species of aquaculture interest, bivalve molluscs constitute a group of major economic importance. Oysters, mussels, scallops and various species of clams, worth millions of dollars, are harvested each year in various part of the world. Both long-term fluctuations in abundance and sudden mass mortalities in bivalve populations have been witnessed. As the development of mariculture has advanced, it has become increasingly apparent that the search of mass mortalities causes is primordial. Indeed the microorganisms, viruses and bacteria have been identified as serious pathogens of free-living, cultivated bivalves. But sometimes microorganisms detected among diseased molluscs are not directly involved in the pathological process. Viruses or bacteria may be present because of the high capacity of water filtration in bivalves. In these cases, it is important to determine if the observed organisms are pathogenic to man or not. It is sometimes difficult to perform an exact identification of the agent, but molecular biology techniques as PCR are very usefull tools.

Moreover, there is increasing evidence that enviromnental stress, an man-made water pollution in particular, may lead to debilitation and disease in numerous marine animals including bivalves.

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