Establishing causal link between an infectious agent and mortalities in marine molluscan aquaculture on the example of Bonamia ostreae and Herpèsvirosis in oysters : proposal of a causal grid analysis

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Summary

In marine molluscan aquaculture, infectious agents are regularly cited in literature associated with mass mortalities as emerging diseases, but the causal link is often based on very few arguments. 24 ways to demonstrate causal links from epidemiological, ecological and marine pathology literature feasible for marine molluscs are given. Those criteria were applied to the example of *Bonamia ostreae* for *Ostrea edulis* and Herpesvirus for spats and larvae of *Crassostrea gigas*. Three principles could be applied in marine field for choosing criteria which should be taken in priority: the demonstration must be given at three levels of organization, organ, individual, population, the demonstration must keeps maximum epidemiological criterias, and then mixes experimental and field work. Results shown that the demonstration is done for *Bonamia ostreae* but still difficult and not completely done for Herpesvirus on spats.

Introduction

The study of bivalve mollusc diseases is quite a new subject of surveillance for many countries all over the word, and suffer from many constraints to make a diagnosis. For the French marine mollusc aquaculture the need for the early detection of an emerging disease is obvious after three catastrophic epizootics. In marine mollusc aquaculture infectious agents are regularly cited in literature associated with mass mortalities as emerging diseases. But the causal link is often based on very few arguments, mainly based at organ level after histological and ultrastructural examination. Causal link was studied from medical science from epidemiology and also from epistemology. Many criteria couldn't be applied easily in marine environment. Specific arguments must be developed in order to confirm or not the causal between the detection of a new agent and mass mortalities.

Objectives

The objective of this study is to find practical criteria in case or after massive mortalities of molluscs in order to confirm or not the existence of an emerging disease, after the detection of a "new or exotic agent".

Materials and methods

The criteria were taken from three disciplines, epidemiology (and epistemology)(1,2), ecology (3) and mollusc pathology (4,5,6). The criteria came from a bibliographic synthesis and also from the French experience of situations with massive mortalities linked or not clearly with infectious agents. In this study two examples are given: the case of parasitic disease *Bonamia ostreae* in flat oysters *Ostrea edulis* (OIE listed disease), the case of viral diseases Herpesvirus for *Crassostrea gigas* (not listed in OIE listed disease).

Results

The grid analysis (table 1) is done at three levels regarding the host : at the level of the organ, the individual, the population, and regarding the pathogen and the environment. The link with epidemiological, ecological, or marine pathology causal criteria is given with the alphabetic reference of the way to demonstrate causal link . The time and means usually needed is qualitatively given with the alphabetic reference of way to demonstrate causal link. For the three levels of organizations, 24 ways to demonstrate causal links feasible for marine molluscs were given in table 1. Results for *Bonamia ostreae* (*Ostrea edulis*) and Herpesvirus (*Crassostrea gigas* larvae and spats)for causal criterias is given in the table 2. Whenever a causal criteria is missing it is written in remarks of table 2. Results shown differences between those infectious agents. For Herpesvirus and *Crassostrea gigas* less causal criteria are demonstrated than for the two other situations.

Table 1 : causal criterias for an emerging disease for marine molluscs

Organ	Individual	Population
Ways to Demonstrate Causal links : A. Necrosis or apoptosis associates (near) with the parasite B. Infiltration of haemocytic cells associates with the parasite C. Localization in a vital organ D. Localization in an organ compatible with symptoms (ex digestive gland-lack of reserves) E. Invading the organ (s) such as no compatible with the function(s) of this organ F. No other pathogens present in high quantity in the same organ G. Always same lesions on representative sampling of animals H. Specificity of lesions associates with parasites (inclusions) **References :** *Epidemiological or Medecine criterias* • Biological plausibility, in agreement with biological knowledge or observations (A, B, C, D, E) • Virulence (C,E) • Qualitative association (A,B,C,D,E,F) and quantitative association (G) • No other factor (F,H) • Consistency and reproducibility (G,H) ***Marine pathological criterias:*** • Necrosis and infiltrations are signs of organs sufferings	**Ways to Demonstrate Causal links** : I. High Level of infestation at mortality moment (infrapopulation) J. Low Level of infestation of other parasites (infracommunauty) at mortality moment K. Rapid and High increasing of infestation just before mortalities (kinetic) L. For I, J, K on a representative sampling of animals M. Experimental reproduction with infected animals N. Experimental reproduction with purified parasite O. Effect of changes of host or parasite antibiotherapy, differential filtration, comparison resistant and susceptible animals P. No interaction with environmental factor not controlled or not known **References :** *Epidemiological or Medecine criterias* • Biological plausibility, in agreement with biological knowledge or observations (I,J,K) • Virulence (I,J,K) • Anteriority (K, M, N, O) • Association (all criterias) • No other factor (J and P, or M) • Consistency and reproducibility (L,M,N) • Experimental proof of disease reproduction with two levels (M, N) • Dosis-Effect Relationship (M,**N**) • Changes of manifestation and spreading of the disease with changes of host (M,N,O) • Supressing the factor (O) *Ecological –biological criteria* • Compatibility filter open (all criterias) *Marine pathological criteria*	**Ways to Demonstrate Causal links** : Q. Exposing free and susceptible animals in contaminated areas in order to reproduce the disease R. Cross-sectional studies in different areas and/or historical data on other areas S. Pluridisciplinary descriptive study in the field, rapid and high increasing of prevalence (incidence) just before mortalities (kinetic), with higher level of morbidity and lethality (repeated cross sectional studies) T. Temporal and Spatial studies of the propagation of disease agent and mortalities U. Case-control studies V. Cohort studies W. differential susceptibility of selected (against the pathogen) populations of host exposed in the field X. Analogy with other disease agent **References :** *Epidemiological or Medecine criterias* • Biological plausibility, in agreement with biological knowledge or observations (X, S) • Anteriority (Q, R,S,T,U) • Association (Q, R, S,T,U,V,W) • No other factor (Q, R, S,T,U,V,W) • Consistency and reproducibility on different populations at different periods (Q, R, S,T,U,V,W) *Ecological criteria* • Geographical distribution indicates an association between the disease and causal factor (meeting filter "open" (**R**,T,U,V) • Compatibility filter open (W,S) *Marine pathological criterias* • Reproducing disease in the contaminated area (Q)

Usual practice : Histopathological exam (2 weeks) Ultrastructural exam (2 weeks) In situ Hybridization (if feasible 3 days) Serum sampling and numeration PCR (if feasible 3 days not quantitative) Criteria D feasible in particular cases	• Examination feasible of all organs of animals (I,J ,K) • Examination of the effect of pluriinfestation and of environmental factors (J,O) • Research or selection of resistant strains of animals to the disease (O) • Cohabitation or filtration work between tanks (M, N,O) **Usual practice :** Same diagnosis tool with column 1 Duration of cultivation and experimental work depends of type of infectious agent Experimental work with statistical analysis and only feasible in some laboratories (depends of situation) M usually done, duration not predictible	• Pluridisciplinary descriptive studies including physiologists, geneticians, marine ecologists, physicians, modellers, pathologists (R,S,T,U,V,W) • Research or selection of resistant strains of animals to the disease (Q,W) • Analogy with other agent or with other species (X) • Determination of the spatial and temporal scale of the phenomena in an open environment (T) • Association of the disease agent with sudden **high** level of mortalities or morbidities (all criterias) **Usual practice :** Q easily done, duration not predictable R easily done and rapid U easily done and rapid X rapid but not very consistent T can be done but needs people in the field, accessibility of animals, means S, V, W needs particularly pluridisciplinary and competent team and if possible means (measurements of environmental factors) and needs time

Table 2 : Results for *Bonamia ostreae on Ostrea edulis* and for Herpesvirosis on spats *Crassostrea gigas*

Disease	Level	Yes	No	Remark on demonstration	Total	Remark on causal criterias
Bonamia ostreae on *Ostrea edulis*	Organ Individual Population	7 8 8	1	D not appropriate N with intracardiac inoculates mainly, P known as density, temperature S,T done 20 years ago	7/8 8/8 8/8	All criterias All criterias All criterias
Herpesvirosis larvae C. Gigas	Organ Individual Population	6 7 4	2 1 4	B and H not appropriates O not shown R, U,W, X not shown at hatchery level, difficult to prove not infected status, no analogy with viral diseases on molluscs, For X first detection of viral agent on gigas larvae associates with mortalities, but other larvae species susceptible	7/8 7/8 4/8	All criterias All criterias All criterias
Herpesvirosis spats C. gigas	Organ Individual Population	6 2 1	2 6 7	B and H not appropriates K,L,M,N,O,P, not shown with reproducibility R,S,T,V were done with no significant or reproducible effect, Q, U, W not shown. X compatible with Herpes pathology with other species	6/8 2/8	Anteriority, no other factor, consistency and reproducibility, dosis-effect not shown

Discussion

The causal link between infectious diseases and mass mortalities with marine molluscan is often based on very arguments at the organ level. In order to classify causal links criteria, they were divided by their level of application to the organ, to the individual and finally to the population. We could also imagine, in future, molecular arguments or cells arguments.

It is known, since the work of Popper that the list of causal links could be infinite. But the main idea of this work, is at least to obtain some of them for demonstrating the link between an infectious agent and mortalities for molluscs. The first principle is to obtain causal link at the three levels of organization (organ, individual, population). The second principle is to obtain mainly principles defined as Epidemiological or medecine criteria defined in table 1 with the quality required by marine pathological criterias. The third principle is to obtain results from observation (field studies) **and** experimental work. Three points explained that we are more concerned by the research of a cause necessary and sufficient. Emerging diseases in molluscs aquaculture are mainly due to transfers of different species of molluscan, and new epizootics kills more than 50% of animals, and the detection of mortalities begins at 20% in 15 days. *Bonamia ostreae* is a parasitic disease of the flat oyster *Ostrea edulis*, in France, but a quite similar parasite Bonamia spp. was detected on *Tiostrea chilensis*, in Chile. The disease was introduced in Brittany with a stock of infected oysters. Massive mortality of the native stock of flat oysters appeared some months after introduction. The grid criteria of causal link with massive mortality could be applied to *Bonamia ostreae* on *Ostrea edulis* for most criteria : by example at the cell level, intra cellular parasitism of haemocytes are increasing, to the destruction of the cell, and at population level, cohort studies show increasing level of prevalences preceding mortalities in contaminated environment compared with non contaminated one, without parasite and mortalities (5). The experimental transmission of the disease can be done. By comparison the link between summer spats mortalities with Herpèsvirosis is less clear, probably because there's interaction with many other factors and because of the diagnosis tool used for detection is by PCR, and because this is a viral disease (Polymerase Chain Reaction)(6). The causal link can also evolves in space and time. There's no more massive mortalities of *Ostrea edulis* in France, because densities are lower and zootechnical practices have changed. The scale of distance is probably important also with summer mortalities of spats, because the distribution of mortalities seem patchy and randomness at some meters of distance. The problem of the heterogeneity of marine coasts has to be taken into account for multifactorial studies. For larvae, the demonstration is more successful (6). A clearful synthesis of infectious agent should make the situation clearer in order to permit safety international exchanges. The infectious agent examined should not to be only OIE listed diseases but also other agents with unknown real pathogenicity and should permit to exhibit lacks of epidemiological or experimental research.

References

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