
Searching for evidence of pathogen exchange in aquatic environments: limits of epidemiological tools

Nacho de Blas

Laboratory of Fish Pathology
University of Zaragoza (Spain)



Does pathogen exchange
exist from aquatic farms to
wild populations?

Does pathogen exchange
exist from wild populations
to aquatic farms?

Nantes, February 2005



There are two important questions. But answers can be very different. Most of textbooks, academic staff and researchers say "Yes, it's evident" to both questions. An ecologist would say "Of course, yes" to the first one and "Perhaps" to the second one. And a fish farmer would say "Probably no" in the first case and "Probably yes" to the second one.

Does any evidence exist related with pathogen exchange between aquatic farms and wild populations?

Which is the risk of pathogen exchange for every mechanism of transmission?

Nantes, February 2005



Perhaps, it would be necessary to reformulate the question “Does any evidence exist related with pathogen exchange between aquatic farms and wild populations? ” as following “Which is the risk of pathogen exchange for every mechanism of transmission?”.

Approaches to search evidence

- Detect the pathogen in wild populations close to infected farms
- Compare prevalences of wild populations: close to infected farms versus areas without farms (or with non-infected farms)
- Likelihood of infection of a free-pathogen wild population exposed to a new infected farm

Nantes, February 2005



In order to answer the first question, researchers had normally used three different approaches:

- The first one doesn't reach too much evidence, because statistical significance is low or doesn't exist and it doesn't respect some causality criteria: strength of association and time sequence (When does the pathogen appears first? In wild population or in farmed animals?).
- In the second design (is like a case control study) it is possible to get more statistical evidence, and it's useful in order to determinate the continuous transference of pathogen from fish farms to environment, and to demonstrate pathogen exchange if prevalence is zero in control population (sentinels). The problem arises when the pathogen is detected in non exposed wild populations or when infections are detected at very low prevalence in wild populations. Another problem is that we don't know the initial infection status of studied populations.
- The last option is equivalent to a cohort study (conceptually), and only in few cases it is possible to carry out. Also could be difficult to demonstrate pathogen exchange if prevalence of infection is low in wild populations.

Epidemiological limitations

- Definition of studied populations
- Quality of diagnostic tests
- Sampling methodology
- Characterization of pathogen

Nantes, February 2005



About the epidemiological limitations of this kind of studies, we think that the main limitations are the next ones:

- Definition of studied populations
- Quality of diagnostic tests
- Sampling methodology
- Characterization of pathogen

Definition of studied populations

■ Farmed animals:

- Population size well known
- Clear compartments (age, sex, species...)
- Easy to observe and to collect samples
- Stressed animals
- Control food intake

■ Wild populations:

- Population size unknown (limits, migrative movements,...)
- Mixed populations
- Difficult to observe and to collect good samples
- Variable stress
- Partial knowledge of feeding habits

Nantes, February 2005



The studied populations are two:

•Farmed animals: it is easy to detect infection, because is a very controlled population, with lot of factors under control (input and output of animals, mortalities, structure of the population, food administration and so on). The animals are more stressed and cultured at high densities and for these reasons the prevalence of infection is higher when the pathogen is present.

•Wild populations: we unknown lot of thing of these populations: the population size, the limits (when a population begins and when it finishes) and the structure (lot of species can be present). Normally the level of stress is low, so usually the prevalence of infection is low.

Quality of diagnostic tests

- Sensitivity and Especificity
- Is PCR the perfect technique?
- Evaluation of complete protocols:
 - Sampling procedures
 - Pooled analysis
- Low Apparent Prevalence

Nantes, February 2005



Everybody that works in epidemiological studies knows (or must be know) concepts about sensitivity and especificity and its implications in the final results. But in most of the cases we trend to estimate our diagnostics as perfect.

This trend is more evident with the generalized use of PCR, and we usually consider that this technique could be highly sensible (even in some cases the level of sensitivity is given as cells by grams) and completely specific. But it is necessary to evaluate the complete diagnostic protocol, considering the sampling procedures (selection of animals, target tissues, possible interference with fish tissues, conservation of samples, transport, processing of batches or pools of samples...) than they could compromise the sensitivity of the final results.

So in this conditions we can get a low apparent prevalence, but really the true prevalence could be higher.

Sampling methodology

- Easier in farmed populations
- Typical sample size insufficient for wild populations
 - Stratified sampling
 - Increasing sample size to detect low infection (or to estimate prevalences)
- Consider quality of diagnostic test

Nantes, February 2005



In farmed population is easy to sample, and Official Recommendations are valid in this cases (150 animals in order to detect infection/disease over 2%, and 60 animals for 5%), since usually we work with homogeneous populations with detectable prevalences.

But perhaps in wild populations these sample sizes are not enough, due to population structure (different susceptibility by age and specie) added to lower prevalence. So it should be necessary to design a stratified sampling, and/or to use higher sample size in order to detect infection or to estimate prevalences.

Remember to use the sensitivity and specificity of the diagnostic test to adjust the final sample size.

Characterization of pathogen

■ Is the same pathogen present in both populations?

- Genetic diversity of the pathogens
- Current advances in Molecular Biology
- Molecular Epidemiology approach

Nantes, February 2005



Actually is completely necessary to demonstrate that it is the same pathogen, not only the same genus and specie, also the same genetic strain. The great advances registered in the last years on Molecular Biology allow us the availability of news techniques to solve this question.

So the PCR techniques and nucleotide sequencing analysis are very powerful tools that we can apply to large scale epidemiological studies to search evidence of pathogen exchange.

Mechanisms of transmission

■ Direct contact

- **Farm to wild:** escapees, restockings,...
- **Wild to farm:** wild broodstocks or larvi

■ Food

- **Farm to wild:** viscera, sewage of food processing plants,...
- **Wild to farm:** raw products from fisheries, plankton,...

■ Water

Nantes, February 2005



But, our aim is not only to demonstrate exchange of pathogens between farmed and wild populations; for example, to demonstrate the absence of exchange should be very interesting but it is more difficult.

Future studies in this area must be targeted to establish the likelihood of exchange of the different mechanisms of transmission in both senses (farmed to wild, and wild to farmed) in order to provide exact information for further Risk Analysis.

So, in few words, we should have to consider the role of the next mechanisms:

- **Direct contact:** from farm to wild (escapees, restocking practices,...) or from wild to farm (use of wild broodstocks or larvi)
- **Food:** from farm to wild (consume of unprocessed viscera, sewage of food processing plants,...) or from wild to farm (use of raw products from fisheries as food, plankton,...)
- **Water transference**

Of course, the weight of this mechanisms can be very different

Practical example: WSSV

- **Origin:** crabs in China Sea
- **Wild to farm:** wild broodstock used in farming system of *Penaeus monodon*
- **Virus evolution:** China, Taiwan & Vietnam strains less virulent than Thailand strains
- **Farm to wild:** Ecuador and Latin America, *Litopenaeus vannamei* infected and lot of wild crustaceans

Nantes, February 2005



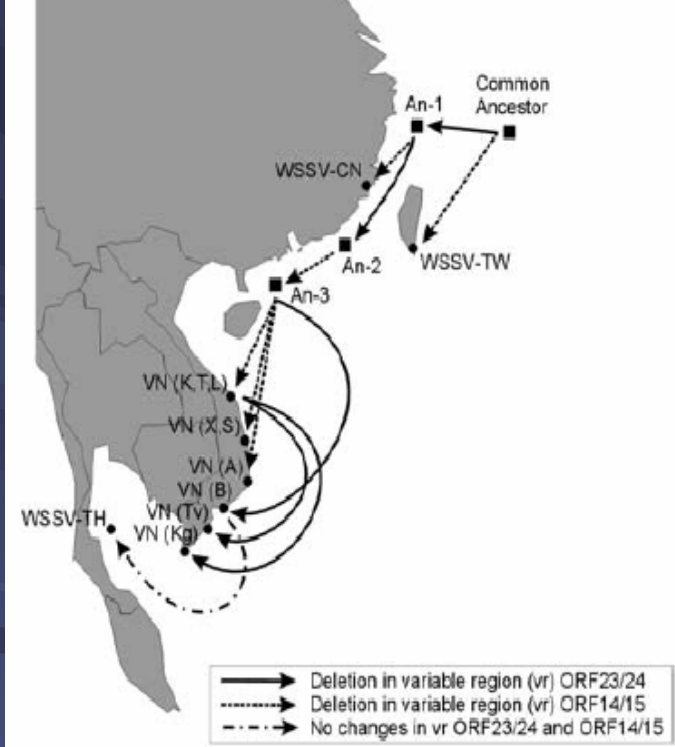
Now, we are going to show a practical example related with the emergence of White Spot Disease caused by WSSV (White Spot Syndrome Virus).

The original virus seems that appears in China Sea in crabs and it is introduced in shrimp farms due to use of wild broodstocks of *Penaeus monodon*

The virus had evolved in the last years and it exists a genetic deletion that increments the virulence (Thai strains).

In 1999 appears the virus in shrimp farms in Ecuador (but affecting to another specie, *Litopenaeus vannamei*), and wild populations of this shrimp and others (*L. stylirostris*) and lot of crustaceans (crabs,...) result infected.

Now it is a vicious circle, with a difficult solution.



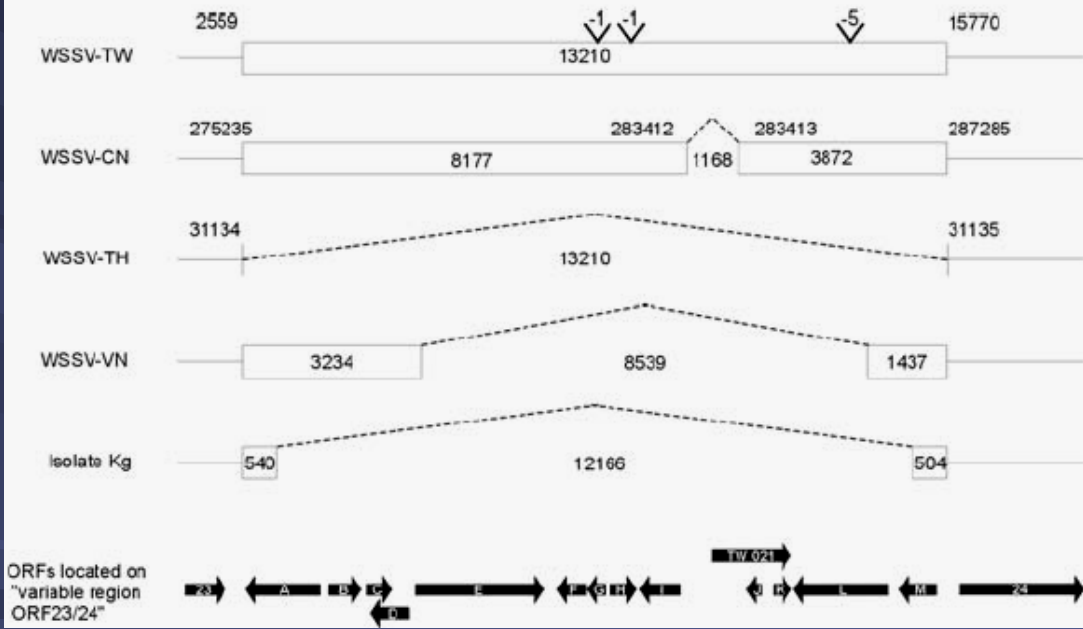
Nantes, February 2005

Just Vlak, ASEM workshop, Barcelona 2004



In the map we can see the origin of the common ancestor of WSSV and its migration associated with animal movements and tidal currents from China and Taiwan to Vietnam and Thailand.

Variable region ORF23/24



Just Vlak, ASEM workshop, Barcelona 2004

Nantes, February 2005



Genetic studies of the region ORF23/24 realized by Laboratory of Virology (University of Wageningen, The Netherlands) show a deletion associated with an increment of the virulence of the strains.

Shrimps:

Litopenaeus vannamei
L. stylirostris
Penaeus monodon
P. japonicus
P. chinensis
P. indicus
P. merguensis
P. penicillatus
P. setiferus
P. aztecus
P. duorarum
P. setiferus
Squilla sp.

Crayfishes:

Pacifastacus leniusculus
Procambarus clarkii
Cherax quadricarinatus
Cherax destructor
Astacus astacus
Astacus leptodactylus
Orconectes limosus
Macrobrachium rosenbergii

Lobsters:

Panulirus ornatus
P. versicolor
P. longipes
P. penicillatus

Crabs:

Calappa lophos
Pornunus sanguinolentum
Charybdis grartulata
Charybdis feriata
Callinectes sapidus
Scylla spp.



More than 300 species of crustaceans show receptivity to the virus, and some of them are susceptible to suffer the disease. This huge range of hosts is one of the keys that explain the exchange of pathogens between wild populations and farmed species.

For the purpose of the *Aquatic Code* of the OIE, all decapod (Order Decapoda) crustaceans from marine, brackish water, or freshwater sources are potential hosts for white spot disease (http://www.oie.int/eng/normes/fcode/A_00063.htm).

Thank you

Nantes, February 2005

