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Acquisition of susceptibility to Vibrio penaeicida in Penaeus stylirostris postlarvae and juveniles

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Abstract:

Vibriosis is a major disease problem in shrimp aquaculture, affecting all developmental stages, from larvae in hatchery tanks to juveniles and broodstock in growout ponds. However, bacterial strains responsible for vibriosis in the successive stages are usually considered to be different, and virulence specificity has been reported both at the species and at the stage levels. The so-called « Syndrome 93 » is a seasonal juvenile vibriosis caused by *Vibrio penaeicida* which affects *Penaeus stylirostris* in growout ponds and broodstock tanks in New Caledonia. This pathology does not cause any mortality in hatchery or nursery phases. An experimental infection design using balneation of postlarvae and early juveniles in *V. penaeicida* suspensions was used to evaluate the developmental stage at which shrimp become sensitive to this vibriosis. We demonstrated that the acquisition of susceptibility to this pathogen is very sudden and correlated with the acquisition of the definitive rostral formula, and from this draw conclusions regarding virulence mechanisms of *V. penaeicida* in *P. stylirostris.*

Introduction

Vibriosis is a major disease problem in shrimp aquaculture, causing high mortalities and severe economic losses in all producing countries (Brock and LeaMaster, 1992; Lightner, 1988 ; Mohney et al., 1994). It is most often considered as an opportunistic pathology in shrimp, but a primary disease caused by highly virulent strains of Vibrio sp. has also been reported (de la Peña et al., 1993). The major genospecies causing vibriosis in shrimp are Vibrio harveyi, V. alginolyticus, V. parahaemolyticus, V. anguillarum (Lightner, 1988 ; Lightner, 1996 ; Jiravanichpaisal et al., 1994). These pathologies occur at all rearing levels, from hatchery tanks to growout ponds. However, specificity has been reported both at the species and the stage level, so strains responsible for larval and juvenile vibriosis are considered to be different, even when belonging to the same species. Different strains are responsible for larval and juvenile vibriosis in Penaeus vannamei in Ecuador, (Mohney et al., 1994). Prayitno and Latchford (1995) demonstrated also both species and stages variations in susceptibility of crustaceans to infection by a Vibrio harveyi-related organism. Sahul Hameed (1995) demonstrated a species-specificity in the susceptibility of three Penaeus species to a Vibrio campbelli-like bacterium.

From 1993, shrimp aquaculture in New Caledonia, based on the closed cycle of captive Penaeus stylirostris in a semi-intensive farming system, has been affected by a seasonal pathology causing mass mortalities that has been named « Syndrome 93 » (Goarant et al., 1996). New Caledonia has a tropical oceanic climate with a hot season from November to May. Mortality episodes frequently occurred during the transition seasons from May to June and from September to October. Moribund shrimp displayed various clinical signs including erratic swimming, lethargy and weakness. High numbers of bacteria belonging to the genus Vibrio are systematically isolated from diseased shrimp haemolymph, revealing a bacterial septicemia. Based on phenotypic and genotypic (ribotyping (Koblavi et al., unpublished data)

and DNA/DNA hybridization (Berthe et al., unpublished data)) studies, the species involved in the « Syndrome 93 » belong to the genospecies Vibrio penaeicida, V. alginolyticus, V. harveyi and V. nigripulchritudo. Since 1994, V. penaeicida strains were most frequently isolated, and their very high pathogenicity was demonstrated in an experimental infection design of Penaeus stylirostris (Le Groumellec et al., 1996).

The purpose of this study was to determine, using experimental infections with the Vibrio penaeicida strain AM 101, the stage at which shrimp become sensitive to this juvenile vibriosis.

Material and methods :

Animals : Postlarvae and juvenile Penaeus stylirostris used in the experiments originated from the experimental Station d'Aquaculture de Saint Vincent. Challenged postlarvae were aged between 17 and 67 days from the first postlarval stage named « PL1 ». Experimental groups were characterized by their mean postlarval stage, calculated as the average of the postlarval stages of 30 to 60 animals at day zero of the infection (see Table 1 : Le Balle, V., unpublished data). There are nine postlarval stages in Penaeus stylirostris, so definitive rostral formulae were considered « PL10 » for calculating mean post larval stages.

Table 1 : Rostral formulae and postlarval staging (Le Balle, V. unpublished data).

Rostral formula	Postlarval stage	Value considered for calculating mean postlarval stage
1/0	PL 1	1
2/0	PL2 and PL3	2.5
3/0	PL4 and PL5	4.5
4/0	PL 6	6
4/1	PL 6	6
5/0	PL 7	7
5/1	PL 7	7
5/2	atypical PL 7	7
6/0	atypical PL 8	8
6/1	PL 8	8
6/2	PL 8	8
7/2	PL 9	9
7/3	PL 9	9
7/4	atypical PL 9 or atypical definitive	9.5
8/3	typical definitive rostral formula	10
8/4	atypical definitive	10
9/3	atypical definitive	10
9/4	atypical definitive	10

Experimental infections : Vibrio penaeicida strain AM 101 was isolated in 1995 from a « Syndrome 93 » diseased juvenile shrimp (Costa et al., 1996). It was cultured in Marine Broth 2216 E (Difco Laboratories) for 24 hours with constant shaking at room temperature. These conditions usually allowed the cultures to reach the late exponential phase. Postlarvae were acclimatized at least 24 hours before infections in a temperature-controlled room (26-28°C). They were individually counted , rinsed with sterile filtered sea-water and placed in one-liter experimental beakers filled with sterile filtered seawater (salinity 33-37‰). Each trial was conducted over a 48-hour period in the temperature-controlled room and included four treatments : Two treatments representing the « Infected » units :

- Addition of 10 ml of a pure 24-hour culture broth per liter (treatment named « Pure »).

- Addition of 10 ml of a 10-fold diluted 24-hour culture broth per liter (treatment named « 1/10 »).

Two treatments representing negative controls :

- Addition of 10 ml of sterile Marine Broth per liter (treatment named « ZoBell »).
- No addition (treatment named « Control »).

All treatments were conducted in 15-postlarvae replicates. Airlift hoses were used for aeration. Postlarvae were fed on crushed Nippaï® (pelleted complete diet for shrimp) ad libitum 2 to 3 times a day. The surviving postlarvae in each beaker were counted at the end of the 48-hour trial period. The concentration of the culture broth was determined a posteriori by plate counting on Marine Agar (Difco Laboratories) serial dilutions of the pure culture.

Positive control : Ten acclimatized juvenile Penaeus stylirostris (5-15 g) were injected intramuscularly with 50µl of the same 24-hour culture broth 1000-fold diluted. Postlarval trial results were taken into account only if all positive control juveniles died within a 36-hour period.

Lastly, in order to evaluate the survival of juveniles in the same experimental design, 5 replicates of 7 juveniles (2.5 g each) in 3-liter beakers were challenged with each of the following treatments : « Pure », « Control » and « ZoBell ». Juveniles reared in earthen ponds aged 67 days since PL1 were acclimatized 24 hours in the temperature-controlled room (26-28° C), rinsed with sterile filtered sea-water and placed in experimental beakers filled with sterile filtered seawater (salinity 33-37‰).

The results of the successive experimental trials were grouped into classes according to their mean postlarval stage (PL4 to 6 ; PL7 to 8 ; PL8 to 8.5 ; PL8.5 to 9 ; PL9 to 9.5 and PL>9.5) for statistical analysis. Data were analyzed using StatView® software.

Results :

Experimental infections were run at concentrations in the ranges $7*10^5$ - $7*10^6$ CFU/ml and $7*10^4$ - $7*10^5$ CFU/ml for « Pure » and « 1/10 » treatments respectively. Mean survival of the trials are shown in Figure 1.

Figure 1 : Mean survival for different postlarval stage classes and treatments. (bars indicate standard deviations)

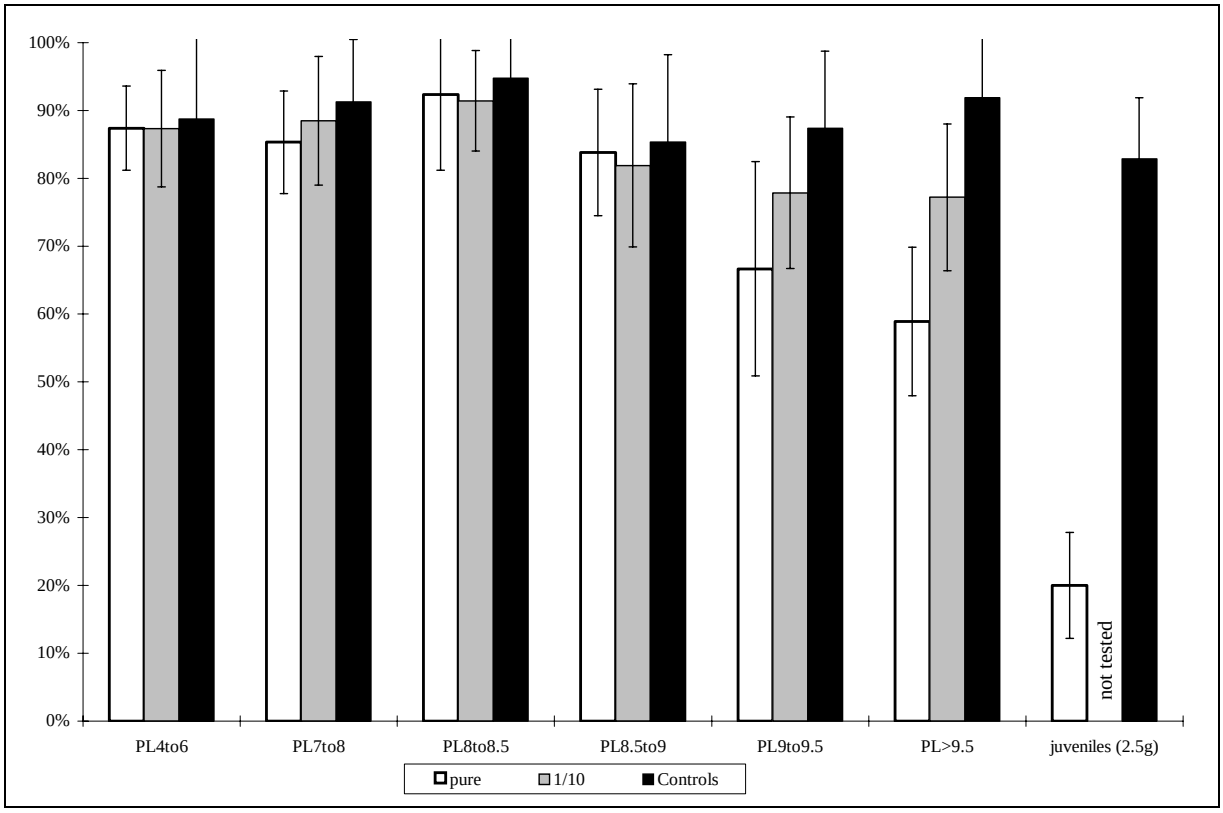

Table 2 : χ^2 test (Chi-square test) comparing survival for each postlarval stage class in « Infected » and « Control » treatments.

	Mean postlarval stage	degrees of freedom	χ^2 test (Chi-square test)	significance level
Infected vs Control	PL 4 to 6	33	0.24	0.627
Infected vs Control	PL 7 to 8	32	2.21	0.137
Infected vs Control	PL 8 to 8.5	38	1.38	0.24
Infected vs Control	PL 8.5 to 9	22	0.40	0.529
Infected vs Control	PL 9 to 9.5	87	45.01	< 0.001
Infected vs Control	PL > 9.5	45	67.93	< 0.001
Infected vs Control	2.5 g juvenile	13	90.79	< 0.001

Table 3 : Chi-square tests comparing survival in different postlarval stage classes over PL9 in « Pure » and « Control » treatments.

Stages compared	Treatment	degrees of freedom	Chi square	significance level
PL 9to9.5 vs PL >9.5	« Pure »	1	14.4	0.001
PL 9to9.5 vs PL >9.5	« Control »	1	0.04	0.84
Juvenile vs PL 9to9.5	« Pure »	1	29.95	< 0.001
Juvenile vs PL 9to9.5	« Control »	1	1.11	0.29
Juvenile vs PL >9.5	« Pure »	1	10.65	< 0.001
Juvenile vs PL >9.5	« Control »	1	1.27	0.26

There was no significant difference between the two negative control treatments « Control » and « ZoBell » : χ^2 test (Chi-square test) run on all data shows that treatments « ZoBell » and « Control » are not significantly different ($\chi^2 = 0.24$, $p=0.626$). They were therefore considered identical and named « Controls ». A χ^2 test (Chi-square test) comparing survival was conducted for each mean postlarval stage class on « Controls » and « Infected » data. It demonstrates that survival is significantly affected by Vibrio penaeicida infection from mean postlarval stage PL9 on ($p< 0.001$, see Table 2). However, susceptibility does not reach its maximum as soon as this stage : survival rates then decreased as mean postlarval stage increased, as demonstrated by Chi-square tests (see Table 3). Even though susceptibility increased among the older juveniles, it can be assumed that Penaeus stylirostris stocks become susceptible to V. penaeicida infection from mean postlarval stage PL9 onward.

Conclusion :

There was no significant effect of experimental infection by Vibrio penaeicida until Penaeus stylirostris stocks reached the mean postlarval stage PL9. From this stage onward, survival is significantly affected by V. penaeicida infection, therefore stocks of animals should be considered as juveniles regarding vibriosis. Mean postlarval stages before PL9 corresponded to stocks where most of the postlarvae do not have a definitive rostral formula. In contrast, later stages corresponded to stocks where some or most of the animals should be considered juveniles because of their definitive rostral formula. Acquisition of susceptibility to V. penaeicida infection appears to be correlated with the acquisition of the definitive rostral formula. Postlarvae may be considered as resistant to V. penaeicida infection, however the animals become sensitive as soon as they reach the juvenile level, characterized by the

definitive rostral formula. The last postlarval molt may therefore correspond to a real metamorphosis including some physiological and/or immunological modifications that would be responsible for the acquisition of this susceptibility. This shift in developmental stage associated with a sudden change in susceptibility is suggestive of the existence of specific receptor structures on shrimp cells that would be involved in one or more virulence mechanism(s) of Vibrio penaeicida to Penaeus stylirostris.

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