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Virus diseases risk-factors associated with shrimp farming practices in rice-shrimp and intensive culture systems in Mekong Delta Viet Nam

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Abstract- In Mekong Delta, viral infection, including white spot syndrome virus (WSSV), monodon baculovirus (MBV), heptopancreatic parvovirus (HPV), infectious hypodermal and hematopoietic necrosis virus (IHHNV) and gill-associated nidovirus (GAV) frequently infect cultured shrimp starting at the postlarvae stage. These viral infections cause high mortality of shrimp and affect the farmer's income. Previous studies mainly focused on the detection, transmission and genetic variation of the pathogens, but few studied the correlation between disease occurrence and other factors, including pond conditions, culture technique and management. Three studies analysed the association of culture factors with the WSSV disease incidence determining risk and potential protective factors in freshwater rice-shrimp systems, and brackish water polyculture and monoculture of black tiger shrimp (*Penaeus monodon*). Studies on the risk and protective factors in the odd of viral diseases in rice-shrimp rotation were lacking, while in 2011 the Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS) caused the great losses of cultured shrimp in Mekong Delta. This study was carried out to describe the culture status of shrimp in Mekong Delta and to identify the risk and/or protective factors that related to disease incidences in black tiger shrimp cultured in 2011. Through interviews, 58 variables collected from 191 farmers (64: rice-shrimp rotation and 127: intensive system) in Soc Trang and Bac Lieu Provinces, Viet Nam. The data were analysed in two steps of variable reduction to increase model stability and binary logistic regression to identify the risk and/or protective factors. The results showed that the risk factors were pond size (entire dataset, rice-shrimp rotation and intensive culture), settling pond (entire dataset), period of pond dry (entire dataset), stocking density first (rice-shrimp rotation), and stocking density second (rice-shrimp rotation). The potential protective factors were the mode of water intake (entire dataset), water level (entire dataset and rice-shrimp rotation), water quality parameters before stocking (entire dataset), and fry test (rice-shrimp rotation).

Keywords: *Penaeus monodon*, virus diseases, protective factors, risk factors

I. Introduction

Black tiger shrimp, *Penaeus monodon*, was the main cultured species in Asian countries, contributing to more than 90% of black tiger shrimp production in the world. The intensification and global market integration brought both higher productivity and disease problems to the culture industry (Bondad-Reantaso et al., 2005). Previous studies have studied various facets involved in black tiger shrimp such as biological and physiological characteristics (Babu et al., 2001; Castillo et al., 2003; Teikwa and Mgaya, 2003; Tu et al., 2006), nutrient and nutrition requirement (Liao and Liu, 1989; Shiao and Cho, 2002; Chen, 1998; Coman et al., 2011), culture and management technique (FAO, 2007), pathogens and immune responses (Cowley et al., 2000; Hettiarachchi et al., 2005; Mohan et al., 2008; Oanh et al., 2008; Sharma et al., 2010) and social economy (Be et al., 2003; Brennan, 2003; Son et al., 2011). The diseases and mortality in shrimps are caused by bacteria (Hettiarachchi et al., 2005; Oanh et al., 2008), parasites (Chakraborti and Bandyopadhyay, 2010; Chakraborti and Bandyapadhyay, 2011), fungi (Khoa et al., 2004) or virus (Chayaburakul et al., 2004). Viruses were considered to be the most serious pathogen in tiger shrimp because of the large mortality and the hard to perceive exterior or histological signs (Ambipillai et al., 2003; Flegel et al., 2004). White spot syndrome virus (WSSV), monodon baculovirus (MBV), heptopancreatic parvovirus (HPV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), gill-associated nidovirus (GAV), and recently the acute hepatopancreatic necrosis syndrome (AHPNS) have affected the production of cultured shrimp (Cowley et al., 2000; Cowley et al., 2002; Chayaburakul et al., 2004; Turnbull et al., 2005; Mohan et al., 2008).

In the Mekong Delta, the WSSV, MBV, YHV and GAV frequently infect to both cultured shrimp and post-larvae (Oanh and Phuong, 2005; and Oanh et al., 2008). The virus occurrence in shrimp farming leads to high mortality and economic losses (Son et al., 2011). Previously, studies mainly focussed on the virus detection, transmission and genetic variation studies (e.g. Peng et al., 1998; Corsin et al., 2001; Sritunyalucksana et al., 2001; Dieu et al., 2004; Tien et al., 2008; Hoa et al., 2011). However, few studies have reported on the relationship between disease occurrence and other factors, including pond conditions, culture technique and management (Hettiarachchi et al. 1999; Corsin et al., 2001; Tendencia et al., 2011). These authors determined risk and potentially protective factors in freshwater rice-shrimp systems, brackish water polyculture and intensive monoculture of *Penaeus* shrimp. However, an effort on identification of the risk and protective factors that related to the odd of virus diseases in black tiger shrimp cultured in rice-shrimp rotation were lacking, while in 2011 the early mortality syndrome, i.e. AHPNS increased the losses of shrimp cultured in Mekong Delta. The present study was carried out to describe the status of shrimp cultured in two provinces of the Mekong Delta and to identify the risk factors as well as the potential protective factors associated with disease incidences in black tiger shrimp cultured in 2011.

II. MATERIALS AND METHODS

A. Sampling and data collection

This study was performed in Soc Trang and Bac Lieu provinces (Viet Nam) from February to July 2011. A total of 191 black tiger shrimp farming households were interviewed, among whom 64 were in rice-shrimp rotation and 127 in intensive system. The interviewed households were listed as having experienced heavy mortality by the local governmental officers and local cooperative staff. The main data of disease occurrence and system production were collected on-farm with a structured questionnaire addressing farm owners, managers or technicians. The questionnaire was pretested with 10 extraneous farmers and then revised based on the feedback. The questionnaire addressed 9 classes of variables including site description, period of culture, pond preparation, water management, fry quality, culture method, feeds and other inputs, diseases and biosecurity measure.

B. Statistical analysis

The data were analysed following the method described by Tendencia et al. (2011). A total of 58 factor variables were binary. Data analysis was carried out in two steps with SPSS.17: variable reduction to increase model stability followed by binary logistic regression. The occurrence of virus disease was used as the dependent variable. The variables were analysed for the entire dataset and for the two subsets of rice-shrimp rotation and intensive culture.

C. Variable reduction

Spearman's rho correlation analysis was applied to identify relationships of all variables with disease incidence. Variables with non-significant (*P>0.1*) univariate slope parameter were not used in the next to increase model stability. The remaining variables were used in the binary logistic regression.

D. Binary logistic regression

Binary logistic regression's backward stepwise method was applied to determine the predictors that relate to the disease occurrence, i.e. mortality. The backward stepwise logistic regression achieves step by step statistical fit of the model with the smallest possible log-likelihood ratio. The negative log-likelihood ratio characterises the relationship between the dependent and independent variables and the R^2 value describes the strength of the relationship between the variables. The classification ratio (CR) represents the percentage correctly classified cases, either 0 or 1, by the model. For each step, the software indicates the model fit. The model with the best combination of low negative log-likelihood value, R^2 value close to one, and high CR was retained for each of the datasets.

The multicollinearity diagnostics from the linear regression analysis was used to assess if the model parameters were biased. In case of multicollinearity, the procedure was repeated replacing a specific factor with a correlated independent factor of the same class to check the contribution to the explanation of the variability.

III. RESULTS

Status of shrimp farming in rice-shrimp rotation and intensive culture systems in 2011

All rice-shrimp rotation systems were practised on land originally used for agriculture. The land where households practiced intensive system was originally used for agriculture (80%), mangrove (18%) or salt production (2.4%). The average farm area (including culture ponds and settling ponds) for rice-shrimp rotation and intensive shrimp culture were 1.3±1.9 and 2.9±5 ha, respectively. The settling ponds were used to settle the water before supplying to culture ponds. The proportion of households that confirmed to use a discharge water treatment pond was high: 100% and 95% in rice-shrimp rotation and intensive culture system, respectively.

Pond preparation and water quality parameters tests were done before stocking in both systems. The majority of the farms used the same canal for supply and discharge of water: 64% and 78% in the rice-shrimp rotation and intensive shrimp system, respectively. In the rice-shrimp rotation system, most of the farms (94%) removed the rice straw before preparing the ponds. In intensive shrimp farms, the pond preparation methods were applied step-by-step subsequently: removing sludge, filtering supply water, observing water colour (i.e. estimating algal density) and applying bio-products to the ponds. More intensive farms (86%) purchased postlarvae (PL) from outside of the province, compared with the rice-shrimp rotation (59%); the values for using PLs tested for external pathogen infection were about identical: 88% and 59%, respectively. The PLs were stocked three times during the culture period in the rice-shrimp rotation system at the density of 14.2, 14.5, and 22.5 PL/m^2; shrimp harvesting was done at outlet gate during each high tide. The intensive culture system was stocked three times a year at the density of 16, 14.7, and 12.9 PL/m^2.

Commercial pelleted feed was used in both cultured systems. Supplemental components such as biological products (100% and 87%), minerals (61% and 76%), nutrient utilization (21% and 45%), and antibiotics (16% and 32%) were added to the feed prior to or during the rice-shrimp rotation and intensive shrimp system, respectively. The rice-shrimp rotation farms did test water quality less frequently (40%) compared with the intensive farms (68%). In both culture systems, diseases caused by WSSV, soft shells, yellow head, hepatopancreas diseases were recorded during 2010 and 2011.

The rice-shrimp rotation had lower average variable costs (43±29.6 million dong/ha/crop) than the intensive shrimp farms (166±166); the variation was large 2.5~150 and 4.2~800 million dong/ha/crop, respectively. The gross margins ranged from -125 to 171.4 (average 0.3±51.1) and from -530 to 1,600 (average 0.3±308.2) million dong/ha/crop for rice-shrimp rotation and intensive shrimp systems, respectively.

Variable reduction

In the rice-shrimp rotation system, pond size, period of pond dry, daily recording, density at 2nd stocking, AHPNS and MBV free, other kind of nutrients, and AHPNS occurrence were positively associated with diseases occurrence, while significant negative coefficients were obtained for sludge removal, water level, alkalinity during culture, testing fry, volume water exchange, diseases occurrence, other farms nearby, crab fence, and having the same inlet and outlet. In the intensive culture systems, total land area, number of ponds, pond size, settling pond, pond rinsing, pH during culture, density 1st stocking, occurrence of AHPNS, and crab fence were positively associated with diseases, while significant negative coefficients were found for the correlation with province, original land use, month of 1st stocking, water purifying method, and period of water exchange. Though classed as significant the coefficient were low (<0.2) for screening (in intensive system), daily recording (in rice-shrimp rotation system), testing pH (in intensive system), density of 1st stocking (in intensive system), and having a crab fence (in intensive system) (Table 1).

Binary logistic regression

The unstandardized coefficient (ß) of the different variables retained in the univariate binary regression with disease occurrence is positive for pond size and the length of the pond dry period in the intensive shrimp system. In the rice-shrimp rotation system, positive ß values were found for pond size, and stocking density, but a negative one for water level. A positive value indicates a higher risk of virus disease outbreak, and conversely a negative value refers to a lower risk (Table 2).

The overall CR was higher in the rice-shrimp rotation culture (90% on average), than in the intensive shrimp culture (66%) (Table 3). The CR for the aggregated datasets was intermediate, though including more and different variables (Table 2). No collinearities were observed between variables in none of the models in the analysis of the two dataset.

IV. DISCUSSION

Pond size was confirmed as a risk factor to the odd of disease outbreaks in both rice-shrimp rotation and intensive shrimp culture. This result confirms the findings for the WSSV incidence of black tiger shrimp cultured in monoculture system (Tendencia et al., 2011), but was not found earlier in rice-shrimp rotation (Corsin et al., 2001). The difference may be due to the other geographical environment or the deviating culture practices. The larger pond size probably constraints the management of water quality and shrimp health (Tendencia et al., 2011).

Water level and volume of water exchange were protective factors, as shown by the high negative ß value, in the rice-shrimp rotation system. The high level of water in cultured ponds is considered to reduce the fluctuation of temperature at different time points in a day; a large fluctuation is a stress factor decreasing the response against disease agents in cultured shrimp (Tendencia and Verreth, 2011). Water exchange during the culture period plays an important role in removing feed residues and shrimp excreta. When not removed these materials increase the sludge accumulation at the bottom of the pond, which may stimulate the growth of pathogens. Most rice-shrimp rotation systems maintain a deep ditch around the central platform where the rice is planted. The water depth on the central platform may be limited by the height of dykes and water level in the supply canal.

The period of pond drying was close to be a significant risk factor in the rice-shrimp rotation system. The longer is the period of pond drying, the higher the risk on disease outbreaks, which might be related to soil acidification.

The low classification ratio of the model for the intensive shrimp culture confirms that the risk factors for disease occurrence are numerous. Indeed about 12 variables had a significant correlation with disease outbreaks. In the rice-shrimp rotation system, the testing of fry reduced the risk on disease occurrence, but this effect was not observed in the intensive shrimp farms. This indicates that most households in intensive shrimp systems purchased tested fry; this study confirms the interests of using tested fry (Flegel et al., 2004). However, healthy black tiger shrimp can be infected from other pathogen carriers (Lester and Paynter, 1989); therefore other management factors during culture period are also important.

In agreement with previous studies, this study found that stocking density was a risk factor in the incidence of disease (Mohan et al., 2008; Giorgetti, 1989; Gunalan et al., 2010; Tendencia et al., 2011). However, Corsin et al. (2001) reported that stocking density was not a risk factor of WSSV-infected *Penaeus* shrimp.

Settling ponds are supposed to play a role in biosecurity for aquaculture by reducing potential pathogens (bacteria, parasites, fungi and virus) and depositing organic matters or solids before supplying the water into culture ponds. In this study, the presence of settling ponds was positively related to disease occurrence in the intensive shrimp culture system, while the rice-shrimp rotation system comprised no settling pond. Tendencia et al. (2011) reported that settling ponds had a protective effect; however most of these ponds used water prepared through the 'green-water' principle, controlling the microalgae in the water. Thus, a settling pond needs to be managed according to specific principles to reduce the risk of disease occurrence.

In our study, daily recording was identified as a significant factor in the rice-shrimp rotation system only, which might again be due to the high frequency of daily recording at the intensive shrimp farms. Daily recording allows the managers to take adjusting measures timely.

V. CONCLUSION

This study provides information on the general status of black tiger shrimp cultured in Mekong Delta in the year 2011. The identified risk factors associated to the odd of virus diseases, included pond size, settling pond, length of pond drying, density at first stocking, and density at second stocking. The settling ponds were not managed according to the 'green-water' principle. The potential protective factors were water purifying method, water level, water quality parameters before stocking, and using tested fry.

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Table 1: The average values of the variables and Spearman`s rho correlation coefficients (*r*) and significance level (*P*) of all variables with diseases occurrence. Values in bold were included in the univariate binary logistic analysis.

Class	Factor	Rice-shrimp rotation culture (n=64)		Intensive culture (n=127)	
		R	*P*	*r*	*P*
Site description	Province	-	-	-0.20	**0.02**
	Original land	-	-	-0.25	**0.00**
	Total land area	0.08	0.53	0.19	**0.03**
	Number of pond	0.15	0.24	0.15	**0.09**
	Pond size	0.30	**0.01**	0.23	**0.01**
	Settling pond	-	-	0.24	**0.01**
Period	Month of 1st stocking	-0.09	0.46	-0.16	**0.08**
	Month of 2nd stocking	-0.21	0.25	0.00	0.98
Pond preparation	Period of water settling	-	-	0.20	0.11
	Sludge removal	-0.24	**0.05**	-0.02	0.85
	Pond rinsing	0.13	0.29	0.23	**0.01**
	Number of pond rinsing	-0.17	0.18	-	-
	Pond dry	0.09	0.49	-0.03	0.72
	Period of pond dry	0.24	**0.06**	0.13	0.16
	Water intake	0.07	0.57	-0.13	0.14
	Water intake through filter	0.02	0.90	-0.02	0.85
	Water purifying method	-	-	-0.18	**0.05**
	Water level	-0.65	**0.00**	-	-
	Water colour	-0.06	0.66	-0.02	0.85
	Probiotics	-0.03	0.83	0.08	0.42
Water management	Daily record keeping	0.26	**0.04**	0.07	0.44
	Water quality parameters	0.05	0.72	0.14	0.11
	pH during culture	-0.10	0.65	0.19	**0.04**
	Alkalinity during culture	-0.63	**0.05**	-0.05	0.69
	Salinity during culture	0.62	0.14	-0.08	0.43
	Temperature during culture	-	-	-0.19	0.63
	Water exchange	-0.13	0.31	-0.01	0.87
	Volume water exchange	-0.43	**0.01**	0.13	0.37

	Period of water exchange	-0.06	0.75	-0.35	**0.02**
	Water exchange source	-0.02	0.92	0.16	0.25
Fry quality	Fry size	-0.07	0.58	0.19	0.13
	Fry test	-0.65	**0.00**	0.10	0.24
	WSSV test	-0.13	0.59	0.12	0.20
	YHV test	-0.13	0.59	-0.07	0.45
	AHPNS test	0.41	**0.08**	0.12	0.21
	MBV test	0.57	**0.01**	0.08	0.39
	TSV test	0.29	0.23	0.13	0.16
Culture method	Method fry stocking	0.22	0.08	0.09	0.31
	Density 1st stocking	0.11	0.39	0.17	**0.05**
	Density 2nd stocking	0.32	**0.07**	0.07	0.55
Feed and other inputs	Vitamin C	0.04	0.82	0.13	0.20
	Probiotics	-	-	0.02	0.86
	Other kind of nutrients	0.31	**0.06**	0.05	0.70
	Antibiotics	-0.21	0.22	0.00	0.97
	Regular use probiotics	-0.05	0.69	-0.02	0.82
Diseases	WSSV	-0.19	0.13	0.06	0.47
	AHPNS	0.21	**0.10**	0.24	**0.01**
	YHV	-0.07	0.58	0.14	0.11
	Diseases occurrence	-0.40	**0.00**	0.06	0.54
	Period of all shrimp dead	-0.32	0.21	0.19	0.13
	Diseases treatment	-0.09	0.49	-0.03	0.73
	Waste water treatment	-0.02	0.87	0.16	0.06
	Other farms	-0.37	**0.00**	0.12	0.22
Biosecurity measures	Crab fence	-0.41	**0.00**	0.15	**0.10**
	The high of crab fence	0.22	0.63	0.00	0.98
	The same inlet and outlet	-0.60	**0.00**	0.03	0.74
	Using the same equipment for whole culture area	-0.01	0.93	0.02	0.84

Table 2: The variables included in the final binary logistic regression (BLR) model that best describe virus risk factors associated to shrimp farming practices 2 provinces in the Mekong Delta of Viet Nam, and the unstandardized coefficients (ß) with significance value (*P*).

Factor	Aggregated data		Rice-shrimp		Intensive culture	
	ß	P	ß	P	ß	P
Pond size (ha)	4.11	0.01	16.22	0.05	3.45	0.06
Settling pond	0.60	0.01				
Water purifying method	-1.14	0.01				
Period of pond dry (day)	0.03	0.00			0.03	0.02
Water level (m)	-9.64	0.00	-25.71	0.01		
Check water quality before stocking	-1.62	0.00				
Fry test			-6.49	0.01		
Stocking density first (postlarvae/m^2)			0.32	0.06		
Stocking density second (postlarvae/m^2)			0.35	0.03	0.00	0.08

Table 3: Characteristics of the binary logistic regression of retained Virus risk factors on the complete dataset and on the subsets of rice-shrimp rotation and intensive culture farms (for variables considered in the model see Table 2).

Subsets	-2LL	R^2	Classification ratio		
			0	1	Overall
Aggregated	171.9	0.36	74.4	81.9	78.5
Rice-shrimp rotation culture	22.7	0.64	86.7	94.1	90.6
Intensive culture	148.3	0.18	55.6	74.7	66.1