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White spot syndrome virus (WSSV) risk factors associated with shrimp farming practices in polyculture and monoculture farms in the Philippines

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ABSTRACT

White spot sydrome virus (WSSV) is one of the most important viral disease of shrimp. Several studies to control the disease have been done. Tank experiments identified WSSV risk factors related to the physico chemical properties of the water. A few studies reported pond level WSSV risk factors. This study identifies the risk factors associated with essentially two different farming systems: polyculture and semi-intensive monoculture of *Penaeus monodon*. Data were gathered from a total of 174 shrimp farmers in eight provinces of the Philippines using a structured questionnaire. Forty-seven variables related to pond history and site description, period of culture, pond preparation techniques, water management, culture methods, feed and other inputs, and biosecurity measures were investigated. In the analysis for combined monoculture and polyculture farms, feeding live molluscs was identified as important WSSV risk factors. In addition to feeding live molluscs, sharing of water source with other farms, having the same receiving and water source, larger pond size, and higher stocking density were identified as important WSSV risk factors in monoculture farms. Climate, i.e. stocking during the cold months and sludge removal and its deposition on the dikes were identified as WSSV risk factors in polyculture farms. Protective factors, listed in decreasing significance, were feeding with planktons and high mangrove to pond area ratio, both observed in the dataset with both monoculture and polyculture farms, while only the latter was observed in the dataset for monoculture farms only. No protective factor was observed in the dataset for polyculture farms.
This study confirmed the negative effect of sharing water source with other farms and identified several new factors influencing WSSV infection such as feeding live molluscs increases the risk, while feeding with planktons and high mangrove to pond area ratio reduce the risk.

1. Introduction

Diseases cause devastating losses to the shrimp industry worldwide (Wongteerasupaya et al., 1995). In Asia alone, the industry suggests annual losses of about 4 billion USD. Most of these pandemic diseases are of viral origin and one of the two most lethal is the White Spot Syndrome Virus, WSSV (Flegel, 2009).

There is no treatment for WSSV and prevention is the best way to avoid outbreaks (Menasveta, 2002). Several studies have investigated the effect of disinfectants on WSSV (Chang et al., 1998; Maeda et al., 1998; Balasubramanian et al., 2006). The use of vaccines and immunostimulants to control WSSV has also been explored (Citarasu et al., 2006; Satoh et al., 2008; Sajeevan et al., 2009). Biosecurity measures to exclude the pathogen or reduce its risk have been suggested (Lotz, 1997; Mohan et al., 2005). Measures adopted by the shrimp industry include the stocking of shrimp larvae confirmed WSSV negative with a polymerase chain reaction (PCR), use of disinfectants, closed culture system to reduce water exchange, bird scares, crab fence, foot/tire bath, and limited access to the farm.

Most of the studies done on WSSV risk factors dealt on the carrier organism (Lo et al., 1996; Kanchanaphum et al., 1998; Suppamataya et al., 1998; Otta et al., 1999; Corsin et al. 2001; Hossain et al., 2001; Yan et al., 2004; Liu et al., 2006), transmission (Suppamataya et al., 1998; Corsin et al. 2001; Peng et al., 1998), effect of water physico-chemical parameters (Vidal et al., 2001; Granja et al., 2003; Guan et al., 2003; Rahman et al., 2006; Reyes et al., 2007), and genetic studies (Wongteerasupaya et al., 2003; Dieu et al., 2004; Syed Musthaq et al., 2006). Only a few studies reported risk factors related to pond culture.

WSSV infection has been positively correlated with proximity of the pond to the sea and negatively to ponds closely located within a given cluster (Mohan et al., 2008). Sludge removal, ploughing, liming, complete system dry-out between culture cycles, water filtration through 300 μm mesh screen and phosphorus application through fertilization were reported to reduce risk of WSSV infection (Corsin et al., 2001; Velasco et al., 2002; Mohan et al., 2008). Corsin et al. (2001)

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found no association between stocking density and WSSV infection at harvest. However, Pienado-Guevara and Lopez-Meyer (2006) reported that removal of 40% and 50% of shrimp population with low level WSSV infection may improve survival. Ingestion of infected shrimp or fresh feed and use of commercial feeds have been associated with WSSV infection (Chou et al., 1995, Chou et al., 1998; Corsin et al., 2001).

WSSV still continues to plague the shrimp industry, despite the bulk of information available. Most of this information resulted from experimental or "laboratory"-based studies relating to transmission and to the physicochemical properties of the water. Epidemiology is another approach of understanding a contagious disease. Factors related to pond location and management practices seem to affect WSSV epidemiology but these have hardly been investigated. A survey is appropriate to gather information from a population which is beyond the control of the investigator on an experimental unit. The relationship between a disease and the hypothesized causal factors in a specified population, like between WSSV and farm practices, can be investigated using a questionnaire in a cross-sectional survey.

This study hypothesizes that factors such as pond site and pond management affect the occurrence of WSSV. Using a structured questionnaire, this study aims to identify these risk factors using an epidemiological approach.

2. Materials and methods

2.1. Definition of terms

WSSV incidence is reported when white spots were observed on the shrimp body, with or without laboratory confirmation. Monoculture farms are those farms that culture *Penaeus monodon* only. Polyculture farms culture *P. monodon* with other species such as milkfish, crab, and tilapia in the same pond. Entire dataset refers to the combined monoculture and polyculture farms.

Season denotes to the wet (June–November) and dry (December–May) seasons while **climate** refers to the cold (November to March) and warm (April–October) months. Classification of season and climate were based on Tendencia et al. (2010a). Sludge removal is the removal of the topsoil from the pond bottom after shrimp harvest. Crop rotation is the culture of phylogenetically different species alternately within a given area. Greenwater refers to the innovative technique wherein shrimp are cultured in water coming from another pond where tilapia and other fish species are grown. Other culture refers to culture techniques other than crop rotation and green water. Flushing refers to filling in and draining out of water into the pond during pond preparation before shrimp culture. Animal waste refers to the use of chicken/pig manure or cow dung to fertilize the pond.

Reservoir is the use of a compartment or pond where water is stored/disinfected before use. Settling pond refers to the presence of a compartment or pond where effluent from the culture pond is drained and treated before release into the environment. Fry analysis refers to analysis of fry for abnormalities and diseases like WSSV and luminous bacteria, before stocking. Birdscare are nets or strings installed above the pond to ward off or scare birds. Crab fence are bamboo, nylon screen, or tarpaulin placed on the dike to prevent entry of crabs into the culture pond. Foot and tire baths are containers with disinfectants like chlorine placed in entry points were hands and vehicles can be rinsed or washed before entry to the pond or farm premises. Limited access is the restricted entry to the farm.

2.2. Study population and study sample

A cross sectional study was conducted from November 2007 to December 2008 in eight provinces of the Philippines with reported WSSV incidence, namely: Bulacan, Antique, Aklan, Negros, Cebu, Bohol, Northern Samar and Leyte. The questions addressed the production cycle that was harvested the same year as the interview. Farms that had two croppings per year were interviewed twice.

A total of 174 shrimp farmers were interviewed, 77 were monoculture farms and 97 were polyculture farms. Of the 77 monoculture farms sampled, 34 were interviewed twice and the remaining 33 interviewed only once. The 77 is a census of all monoculture shrimp farms in the eight provinces while the 97 polyculture shrimp farms represent a 10% randomly selected sample of all polyculture farms in the eight provinces. Staff from the local government unit of the different provinces and from a local cooperative assisted in identifying the farms to be interviewed.

2.3. Data collection

The data were gathered using a structured questionnaire that addressed seven classes of variables: (1) pond site which included history and description, (2) period of culture, (3) pond preparation techniques, (4) water management, (5) culture methods, (6) feed and other inputs and, (7) biosecurity measures (please refer to columns 1 and 2 of Table 1). The selection of these variables was based on the different farming techniques implemented by farmers and on the measures suggested to prevent WSSV occurrence as mentioned in the introduction.

The questionnaire was developed in English, and administered in the local language to ensure that farmers would understand all the questions. It was pre-tested or validated with 10 farmers outside the study sample and questions were refined according to feedback from the farmers. The final questionnaire was administered by the main author with the help of the staff from the provincial government unit or from the local cooperative. It was emphasized that answers would be highly confidential, and that correctness of the answers was necessary for the proper analysis of the results. Interviewers were allowed to enter the farm premises in most cases. This allowed the validation of the gathered information. Staffs of the local government unit and of the local cooperative were knowledgeable of the farms' operation, and thus also assisted in the validation.

The respondents were persons involved in farm management: farm owners, managers, technicians and caretakers. If farm owners were interviewed, they referred to the managers or technicians for questions they were unable to answer.

2.4. Statistical analysis

A total of 47 variables (please refer to column 2 of Table 1) were used for the analysis. Data gathered were mostly binary (yes/no) except for months of stocking or harvest, mangrove to pond area ratio (MPR), proximity to the sea or road (km), pond size (ha), water level inside the pond (cm), and number of croppings/year. No scaling was used in the case of the non-binary data. Data analysis was done in two steps: variable reduction to increase model stability and binary logistic regression to identify the protective and risk factors. WSSV incidence was used as the dependent variable that needs to be explained. The procedure was applied to the entire dataset and to two sub-sets of the data: farms into monoculture, and farms into polyculture. All the statistical analyses were done with SPSS®17.

2.4.1. Variable reduction

Variable reduction was done to increase model stability. The correlation of all variables to WSSV was determined using Spearman's rho correlation analysis. Variables with rho correlation significance level $P<0.1$ were selected. Univariate binary logistic regression analysis was applied on the selected variables. Correlation analysis was used for variable reduction to prevent collinearity of variables included in the final model. Variables with univariate slope parameter