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Risk Factors Associated with Acute Hepatopancreatic Necrosis Disease (AHPND) Outbreak in the Mekong Delta, Viet Nam

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Abstract

The Vietnamese shrimp farming industry has experienced massive production losses due to Acute Hepatopancreatic Necrosis Disease (AHPND) caused by *Vibrio parahaemolyticus* (Vp_{AHPND}) since 2011. The objective of this study was to identify factors associated with AHPND occurrence on shrimp farms in the Mekong Delta in Viet Nam. A retrospective cross-sectional study was carried out on shrimp farms in four districts in the Mekong Delta area, Viet Nam from January 2012 to May 2013. Data were collected from 1920 ponds belonging to 1195 farms. Factors related to farm characteristics, farm management, pond and water preparation and management, feed management, postlarval (PL) shrimp, and stock management were evaluated. Multivariable logistic regression analysis was used to determine factors affecting the occurrence of AHPND at the pond and farm levels. The following characteristics were identified as significant farm-level risk factors: (i) having a larger culture area in terms of hectarage, (ii) using the sun-dried sediment method for cleaning pond bottom during the pond preparation process, and (iii) being sited close to other farms using the same AHPND-affected water source. Ponds with the following features were associated with increased risk of AHPND occurrence: (i) water depth of 1.2 m or less, (ii) extremely change of weather events occurring during the first 35 days of culture (DOC) or until the first signs of AHPND, and (iii) use of fertilizers and probiotics for water treatment. Moreover, ponds that were supplied with PL from some specific hatcheries were more likely to be infected with AHPND than were others. On the other hand, the risk of AHPND occurring was reduced in ponds that used minerals and algaecide for water treatment.

Keywords: *Vibrio parahaemolyticus*, acute hepatopancreatic necrosis disease (AHPND), risk factor, farm management

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Introduction

Acute Hepatopancreatic Necrosis Disease (AHPND) has been responsible for huge losses in the shrimp aquaculture industry of many countries since 2010. The disease was first reported in the People's Republic of China in 2010, followed by Viet Nam and Malaysia in early 2011 (Lightner *et al*., 2012), and then Thailand in late 2011 (Flegel 2012). AHPND has a bacterial aetiology (Tran et al. 2013). It is caused by a particular strain of *Vibrio parahaemolyticus* (Vp_{AHPND}), which contain a ~70-kbp plasmid (pVA1) with genes homologous to those coding for the *Photorhabdus* insect-related (Pir) binary toxin ($PirA^{vp}$/$PirB^{vp}$) that induces cell death (Kondo et al*.* 2015; Lee et al. 2015).

The disease typically affects newly stocked penaeid shrimp (i.e. 20–30 days after stocking), including *Penaeus monodon* Fabricius 1798, *P. vannamei,* and *P. chinensis* Osbeck 1765 (NACA/FAO 2011; NACA 2012a). Affected shrimp display signs of lethargy and anorexia, and a pale and atrophied hepatopancreas as a prominent gross lesion, and commonly experience secondary infections with opportunistic bacteria. Shrimp mortality gradually increases, with cumulative pond mortality regularly reaching 100% within a week (Lightner et al. 2012). A polymerase chain reaction (PCR) test for the detection of bacterial isolates that cause AHPND has been available since late 2013 (Flegel and Lo 2014) and nested PCR was reported in 2015 (Sirikharin et al. 2015). However, before the PCR method became available, the diagnostic procedure relied on clinical signs and histopathological finding matching the case definition as given by the AHPND disease card (NACA 2012b).

AHPND was firstly recognized in Viet Nam in Soc Trang Province in late 2010 and continuously occurred and spread to other provinces in the Mekong Delta such as Tien Giang, Ben Tre, Tra Vinh, Soc Trang, Bac Lieu, Kien Giang and Ca Mau provinces in 2011. It caused approximately 50 % reduction of total shrimp production in Viet Nam. The Food and Agriculture Organization of the United Nations (FAO) Project TCP/VIE/3304 was funded to assist the Government of Viet Nam, particularly the Competent Authority on aquatic animal health to achieve a better understanding of the unknown disease that was affecting cultured shrimp and causing significant losses in the Mekong Delta. The objective of this study was to measure the prevalence of AHPND in the Mekong Delta of Viet Nam during January 2012 to May 2013 and understand the pattern of its spread. Moreover, the risk factors related to various farm practices, such as pond preparation, water quality management, PL and stock management were evaluated for their association with the occurrence of AHPND at the pond and farm levels.

Material and Methods

Study area

The study areas were chosen based on information provided by field officers of the Department of Animal Health (DAH). The areas were selected based on the AHPND problem experienced (i.e. AHPND still occurred in the area) and the scale of farm operation in the area. Therefore, districts in three provinces of the Mekong Delta were chosen: Dam Doi District of Ca

Mau Province, Hoa Binh District of Bac Lieu Province, and Vinh Chu and Tran De districts of Soc Trang Province (Fig. 1).

Fig. 1. Four districts included in the study area. Note: DD = Dam Doi District of Ca Mau Province, HB = Hoa Binh District of Bac Lieu Province, VC = Vinh Chu District and TD = Tran De District, both of Soc Trang Province.

Study population and pond selection

Black tiger shrimp (*P. monodon)* and Pacific white shrimp (*P. vannamei*) were the subjects of the study. Both species are cultured in the Mekong Delta area and were experiencing problems with AHPND. A cross-sectional study design was used. The study population consisted of shrimp cultured in ponds during the period January 2012 to May 2013. The list of shrimp farms was provided by DAH officers and used for the sampling frame. To reduce the problem of forgotten or loss of data, the last crop data of ponds in farms that had several crops during the study period were included in the study.

As this study used a retrospective study design, the pond-level case definition used for further analysis was based on clinical signs of AHPND-affected shrimp (presence of a prominent, pale and atrophied hepatopancreas), affected ponds having a cumulative mortality of more than 40 % within 5–7 days after occurrence of clinical signs, and the problem having occurred before 35 DOC. The farm-level case definition was based on the AHPND history of the farm, i.e. an affected farm must have had at least one operation cycle with a pond affected by AHPND from 1 January 2012 until the date of the study.

Sampling size calculation and study type

Sampling size was calculated using the Survey Toolbox software program (Cameron 2002). The prevalence of AHPND in the study areas as reported by Corsin (2012) was used to calculate the sampling size required for each district. Two-stage sampling was used to sample farm and pond data. The 1st stage sampling was randomly selected farms using the method of probability proportional to size. The 2nd stage sampling chose ponds by the simple random sampling method. The number of farms and number of ponds per farm used in this study are indicated in Table 1.

Table 1. Number of studied farms, number of ponds per farm and expected total ponds used in this study

Province	District	Number of farms	Prevalence (%)	Average farm size (# of ponds)	# of farms	# of ponds per farm	Expected total ponds
Ca Mau	DD	2117	29.2	2.67	297	2	594
Bac Lieu	HB	2500	46.9	4.88	332	2	664
Soc Trang	VC	2041	30.4	3.04	304	2	608
Soc Trang	TD	1398	30.4	4	316	2	632

Note: DD = Dam Doi District, HB = Hoa Binh District, VC = Vinh Chu District and TD = Tran De District

Data collection

Data were collected by means of questionnaires administered through face-to-face interviews. Interviewers were trained on how to use the questionnaires prior to carrying out the data collection. The questionnaires were pre-tested to ensure understanding and practical availability of information. Data collected included the following: (i) respondent (farmer) information, (ii) general farm information, (iii) general pond information, (iv) farm characteristics, (v) shrimp species, (vi) culture system, (vii) water management: water and pond preparation, water exchange, (viii) feed, (ix) source of PL shrimp, (x) shrimp age at stocking, (xi) PL stocking density, and (xii) weather conditions during the week prior to disease occurrence (for case) or before shrimp reached 35 DOC (for control).

Statistical analysis

Data were checked for missing values, and mean calculation was carried out for each district. Dummy variables were created for summary of information contained in the record prior to the analysis. Variables were identified by farm and pond levels. Farm-level factors included the following: farm size (number of available ponds), production size (number of active ponds), reservoir area size (measured in hectares), ratio of reservoir to culture area, bottom type, source of water supply, water management system, shrimp species, pond preparation methods, chemical treatment for water preparation, and shrimp health status of nearby farms. Pond-level factors included pond size and depth, water and pond preparation, source of PL, shrimp age at stocking, PL stocking density, feed practice, water quality and exchange rate, other animals in the pond, aerator application, use of probiotics, and weather conditions.

The presence or absence of AHPND at the farm and pond levels was considered the outcome variable. The analytical processes were accomplished using the statistical software package STATA (Version 14.0, Stata Corp, College Station, TX). Multivariable logistic regression was used to evaluate factors associated with AHPND outbreak at farm and pond levels, separately. The assumptions for the logistic regression were assessed (Dohoo et al. 2009). Unconditional associations between each predictor and outcome variables were evaluated using univariable logistic regression. All factors with *P*-value of less than or equal to 0.2 were included in the multivariable analysis. Categorized variables were generated if the relationship between a continuous predictor and log-odds of the outcome was not linear.

The cut-offs were decided at the point of the most change in log-odds when the independent variables changed, and these were incorporated with biological factors prior to the multilevel logistic regression analysis**.** District was considered a potential confounding factor and remained in the model as fixed effect**.** Shrimp species was included as random effect when assessing the farm-level model**.** Farm was included as random effect to account for potential farm effect when assessing the pond-level model**.** The best-fit model was found by a manual backward selection process in which the likelihood-ratio test (LRT) was used to test the significance (*P*-value < 0.05) of subtracting one variable at a time from the models. Interactions effect among significant variables that notice biological synergism plausible were also tested**.**

Results

Survey data were collected from a total of 1254 farms and 2508 ponds in the four study areas (Table 2). We could not complete all of the questionnaires because some farms were no longer in operation. Moreover, some farms had only one pond, so we had only one pond record, which is less than the target number (2 ponds per farm). Therefore, the data collected for farm and pond were 95.3 % and 76.6 % of the goal, respectively.

Table 2. Number and proportion of farms and ponds used for collection of data.

Province	District	# Target farm	# Target pond	#Complete farm	#Complete pond
Soc Trang	VC	309	618	289 (93.5 %)	466 (75.4 %)
Soc Trang	TD	316	632	323 (102 %)	387 (61.2 %)
Bac Lieu	HB	332	664	305 (91.9 %)	586 (88.3 %)
Ca Mau	DD	297	594	278 (93.6 %)	481 (81 %)
Total		1254	2508	1195 (95.3 %)	1920 (76.6 %)

Note: DD = Dam Doi District, HB = Hoa Binh District, VC = Vinh Chu District and TD = Tran De District

Farm-level data analysis

Farm characteristics and farm management

Farm which cultured *P. monodon, P. vannamei* and both species were 84.8 %, 7.1 % and 8.1 %, respectively. Almost half of the farms culturing *P. monodon* (53.6 %) were semi-intensive farms, while intensive farms and improved extensive farms comprised 33.2 % and 13.2 % of the total. respectively.

Farms culturing *P. vannamei* were mainly intensive farms, (90.4 %). Farm managing with open, closed, semiclosed, recycled and mix water management systems were 12.2 %, 53.4 %, 29.5 %, 1.2 % and 3.7 %, respectively.

Descriptive statistics and unconditional associations for each factor

More than half of the interviewed farms (i.e. 78.5 %) experienced AHPND. Prevalence of AHPND in *P. monodon*, *P. vannamei* and both secies farms was 79.2 %, 65.7.0 % and 82.5 %, respectively. Prevalence of AHPND in *P. monodon* farms was 78.5 %, 78.2 % and 86.7 % for intensive, semi-intensive and improved extensive farms, respectively. Prevalence of AHPND in intensive and semi-intensive *P. vannamei* farms was 74.2 % and 78.6 %, respectively. The results from unconditional association analysis are presented in Table 3.

Table 3. Descriptive statistics and unconditional associations for factors with the potential to affect AHPND occurrence in shrimp farms.

	Control			**Case**			
Variable name	**n**	**%**	**Mean (SD)**	**n**	**%**	**Mean (SD)**	***P*-value***
District							<0.001
- Dam Doi (DD)	59	27.8		132	17.0		
- Hoa Binh (HB)	97	45.8		194	25.1		
- Tran De (TD)	45	21.2		198	25.6		
- Vinh Chu (VC)	11	5.2		250	32.3		
Number of culture ponds	211		3.1 (2.9)	774		3.7 (6.0)	0.107
Total culture area (hectare)	211		1.2 (1.1)	771		1.7 (4.6)	0.004
Water reservoir							0.178
Not available	104	49.1		339	43.9		
Available	108	50.9		434	56.1		
Total reservoir area (hectare)	212		0.2 (0.6)	771		0.2 (0.4)	0.481
Ratio of reservoir to culture area	211		0.3 (0.8)	770		0.2 (0.4)	0.033
Source of water supply							0.000
Fresh water (<0 ppt)	0	0.0		3	0.4		
Brackish water (5−15 ppt)	51	24.1		321	41.5		
Sea water (>15 ppt)	142	67.0		405	52.3		
More than one source	19	8.9		45	5.8		
Water management system							0.005
Open	27	12.7		93	12.0		
Closed	85	40.1		442	57.1		
Semi-closed	87	41.1		204	26.4		
Recirculation	3	1.4		9	1.2		
Mixed system	10	4.7		26	3.3		
Water supply and drainage							0.01
Same water inlet and outlet	164	80.4		537	71.4		
Separate water inlet and outlet	40	19.6		215	28.6		
Shrimp culture species							0.735
P. monodon	174	82.1		662	85.5		
P. vannamei	24	11.3		46	6.0		
Both species	14	6.6		66	8.5		
Culture system (*P. monodon*)							0.146
Intensive (>20 PL/m^2)	65	34.8		237	32.8		
Semi-intensive (10−20 PL/m^2)	106	56.7		381	52.8		
Improved extensive (<10 PL/m^2)	16	8.5		104	14.4		
Culture system (*P. vannamei*)							0.724
Intensive (≥ 60 PL/m^2)	34	91.9		98	89.9		
Semi-intensive (< 60 PL/m^2)	3	8.1		11	10.1		
Pond bottom type							0.658
Soil	205	97.2		746	96.8		
Plastic lining (slope or all area)	6	2.8		25	3.2		

Table 3. Continued**.**

	Control			Case			
Variable name	**n**	**%**	**Mean (SD)**	**n**	**%**	**Mean (SD)**	***P*-value***
Cleaning pond with at least one method							0.195
not applied	2	1.0		2	0.3		
applied	210	99.0		771	99.7		
Cleaning pond bottom by flushing							0.32
not applied	100	47.2		335	43.3		
applied	112	52.8		438	56.7		
Cleaning pond bottom by soil removal							0.006
not applied	105	49.5		302	39.1		
applied	107	50.5		471	60.9		
Cleaning pond bottom by sun drying							0.002
not applied	24	11.3		40	5.2		
applied	188	88.7		733	94.8		
Duration of sun drying (days)	152		20.5 (16.8)	459		29.0 (19.8)	0.000
Cleaning pond bottom by ploughing							0.725
not applied	198	93.4		727	94.0		
applied	14	6.6		46	6.0		
Liming pond							0.966
not applied	19	9.0		70	9.1		
applied	193	91.0		703	90.9		
Carricide control program during pond preparation							
not applied	3	1.4		10	1.3		0.894
applied	209	98.6		761	98.7		
- Filtration inlet water (not used)	15	7.1		54	7.0		0.982
Filtration inlet water (used)	197	92.9		714	93.0		
- Chlorine (not used)	104	49.1		428	56.6		0.051
Chlorine (used)	108	50.9		328	45.4		
- Insecticide (not used)	195	92.0		699	92.5		0.817
Insecticide (used)	17	8.0		57	7.5		
- Saponin (not used)	106	50.0		389	51.5		0.708
Saponin (used)	106	50.0		367	48.5		
- Other carricide agent (not used)	171	80.1		612	81.4		0.812
Other carricide agent (used)	41	19.9		140	18.6		
Application of probiotic bacteria during pond preparation							
not applied	157	74.1		562	74.6		0.865
applied	55	25.9		191	25.4		
Water treatment before using							
not applied	18	8.9		117	15.9		0.013
applied	184	91.1		617	84.1		
- Holding water (not used)	28	13.9		171	23.4		0.004
Holding water (used)	174	86.1		561	76.6		
- Chlorine (not used)	139	68.8		501	68.6		0.961
Chlorine (used)	63	31.2		229	31.4		
- Other disinfectant (not used)	170	84.2		637	87.5		0.216
Other disinfectant (used)	32	15.8		91	12.5		

Table 3. Continued**.**

	Control			Case			
Variable name	**n**	**%**	**Mean (SD)**	**n**	**%**	**Mean (SD)**	***P*-value***
Duration water withheld in reservoir (days)	201		11.9 (10.6)	722		10.2 (9.8)	0.026
Feed brand	70 different brands were used, with none showing a significant relation to AHPND outbreak						0.434
Feed broadcast manner							
Hand without feeding tray	61	28.9		343	44.9		0.000
Hand with feeding tray	150	71.1		417	55.1		
Mechanical by automatic feeder	0	0.0		4	0.0		
Feed storage condition							
Cool shaded storage	9	4.4		20	2.7		0.741
On-shelf storage	2	1.0		25	3.4		
Both methods	195	94.6		700	93.9		
Duration feeds stored in farm until using (days)	200		7.5 (3.6)	690		7.1 (3.2)	0.104
Nearby farms using same water source that is affected by or not affected by AHPND							
Not affected	95	49.7		130	17.9		0.000
Affected	96	50.3		596	82.1		

Note: *factors with *P*-value of less than or equal to 0.2 were included in the multivariable analysis**.**

Table 4. Final multiple logistic regression model for factors associated with AHPND occurrence in shrimp farms.

	OR	**SE**	**Z**	***P*-value**	**95 % CI**
- Total culture area (hectare)	1.3	0.10	3.56	0.000	(1.1, 1.5)
- Cleaning pond bottom by sun-dry method	2.0	0.64	2.11	0.035	(1.1, 3.7)
- Nearby farms using same water source that is affected by EMS/AHPND	5.1	0.97	8.46	0.000	(3.5, 7.4)
District					
Dam Doi (DD)			Reference		
Hoa Binh (HB)	0.6	0.13	-2.48	0.013	(0.3, 0.9)
Tran De (TD)	1.5	0.38	1.53	0.126	(0.9, 2.5)
Vinh Chu (VC)	8.3	3.03	5.75	0.000	(4.0, 17.0)
Constant	0.4	0.14	-2.61	0.009	(0.2, 0.8)

Note: OR = odds ratio, SE = standard error, Z = standard normal deviate, 95 % CI = 95 % confidence interval

Multivariable logistic regression model

The final multivariable logistic regression model of factors associated with AHPND cases in shrimp farms in in the Mekong Delta area after controlling for the confounding effect of district is presented in Table 4. Factors with odds ratio (OR) greater than one are interpreted as increasing the risk of having AHPND in shrimp farms. Those factors with OR less than one are considered having protective effect. The prevalence of AHPND varied among districts, the highest prevalence being recorded in Vinh Chu District.

Risk factors that were related to AHPND occurrence include: (i) farm with larger culture area in terms of hectare, (ii) farm using sun-dry sediment method for cleaning pond bottom during pond preparation process, and (iii) farm site nearby other farms and using the same water source that is affected by AHPND. No significant interaction among significant variables was detected.

Table 5. Descriptive statistics and unconditional associations for factors with the potential to affect AHPND occurrence in shrimp ponds.

	Control			Case			
Variable name	**n**	**%**	**Mean (SD)**	**n**	**%**	**Mean (SD)**	***P*-value***
District							
- Dam Doi (DD)	246	36.6		201	16.7		reference
- Hoa Binh (HB)	322	47.9		260	21.6		0.925
- Tran De (TD)	79	11.8		306	25.4		0.000
- Vinh Chu (VC)	25	3.7		437	36.3		0.000
Pond characteristic							
- Pond size (hectare)	670		0.4 (0.3)	1,197		0.5 (0.4)	0.000
- Pond depth (meter)	664		1.4 (0.2)	1,170		1.3 (0.2)	0.000
Water treatment							
- Fertilizer use (not used)	437	65.8		780	66.2		0.881
Fertilizer use (used)	227	34.2		399	33.8		
- Fertilizer type							
- not used	437	66.4		780	67.1		reference
- inorganic	200	30.4		353	30.4		0.916
- organic	12	1.8		25	2.21		0.664
- both	9	1.4		5	0.4		0.037
- Application of any chemical for water quality management							0.045
- not used	98	14.8		136	11.6		
- used	563	85.2		1,040	88.4		
- Mineral application (not used)	52	8.8		66	6.2		0.048
Mineral application (used)	536	91.2		996	93.8		
- Disinfectant application (not used)	244	41.9		392	38.2		0.143
Disinfectant application (used)	338	58.1		634	61.8		
- Algaecide application (not used)	456	78.5		720	73.9		0.040
Algaecide application (used)	125	21.5		255	26.1		
- Pesticide application (not used)	565	97.3		935	97.7		0.578
Pesticide application (used)	16	2.7		22	2.3		
- Probiotic application (not used)	116	20.0		332	33.0		0.000
Probiotic application (used)	465	80.0		675	67.0		
Antimicrobial application							
- not used	406	69.9		716	73.6		0.115
- used	175	30.1		257	24.4		
Postlarval (PL) management							
- Shrimp species							0.000
- *P. vannamei*	121	18.3		111	9.4		
- *P. monodon*	541	81.7		1074	90.6		
- Stage of PL at stocking date	652		12.9 (1.9)	1123		12.9 (2.0)	0.975
- Stocking density (PL/m^2)	663		32.3(29.4)	1184		25.4(21.8)	0.000

Table 5. Continued.

Variable name	Control n	Control %	Control Mean (SD)	Case n	Case %	Case Mean (SD)	*P*-value*
Supply PL source (hatchery)							
- Small-sized hatchery	301	59.0		413	49.5		reference
- Medium-sized hatchery	24	4.71		98	11.8		0.000
- Hatchery A	22	4.3		49	5.9		0.070
- Hatchery B	40	7.8		131	15.7		0.000
- Hatchery C	52	10.2		88	10.6		0.271
- Hatchery D	71	13.9		55	6.6		0.000
Potential diseases carrier							
- Presence of wild animals in the pond							0.393
- absent	476	71.9		879	73.7		
- present	186	28.1		313	26.3		
- Crabs (absent)	545	82.7		1024	86.1		0.049
Crabs (present)	114	17.3		165	13.9		
- Finfish (absent)	621	94.5		1082	91.1		0.008
Finfish (present)	36	5.5		106	8.9		
- Wild shrimp (absent)	617	93.8		1090	91.8		0.117
Wild shrimp (present)	41	6.2		98	8.2		
Water quality							
- Morning pH	269		7.8 (0.4)	521		7.6 (0.3)	0.000
- Afternoon pH	232		8.1 (0.4)	465		8.1 (0.4)	0.022
- Salinity	189		16.8 (7.6)	252		14.0 (7.0)	0.000
Water management							
- Exchange or topping up of water during the first 35 DOC (or until the first signs of AHPND)							
- NO	629	93.6		1157	96.1		0.016
- YES	43	6.4		47	3.9		
- Aerator application (not used)	38	5.8		147	13.0		0.000
Aerator application (used)	619	94.2		984	87.0		
Environmental							
Any unusual climatic events during the first 35 DOC (or until the first signs of AHPND)							
- NO	293	59.1		423	40.8		0.000
- YES	203	20.9		615	59.2		

Note: *factors with *P*-value of less than or equal to 0.2 were included in the multivariable analysis.

Pond-level data analysis

Descriptive statistics and unconditional associations for each factor

Sixty four percent of the study ponds had experienced an AHPND outbreak. The prevalence of AHPND in ponds in Dam Doi (Ca Mau), Hoa Binh (Bac Lieu), Tran De (Soc Trang) and Vinh Chau (Soc Trinang) was 45.0, 44.6, 79.5 and 94.6 %, respectively. Pond which culture *P. monodon* and *P. vannamei* were 87.4 and 12.6 %, respectively. PL was supplied from 222 hatcheries with the frequency of supply ranging from 1–173 ponds. Due to the many categories, it was not possible to analyzed this factor.

We overcame this problem by grouping this factor by frequency of supply, so hatcheries that supplied PL to less than 20 ponds were grouped as small-sized hatcheries and used as the reference group for logistic regression analysis, those hatcheries that supplied PL for 20–30 ponds were classified as medium-sized hatcheries, while those hatcheries that supplied PL to more than 30 ponds were not grouped. The results from the unconditional association analysis are presented in Table 5.

Table 6. Final multivariable logistic regression model for factors associated with AHPND occurrence in shrimp ponds.

	OR	**SE**	**Z**	***P*-value**	**95 % CI**
Pond characteristic					
- Pond depth (0 if >= 120 cm; 1 if < 120 cm)	1.6	0.33	2.48	0.013	(1.1, 2.4)
Water treatment					
- Fertilizers	1.4	0.24	2.20	0.028	(1.0, 2.0)
- Mineral application	0.5	0.16	-2.18	0.029	(0.3, 0.9)
- Algaecide application	0.6	0.12	-2.68	0.007	(0.4, 0.9)
- Probiotic application	1.5	0.33	2.01	0.044	(1.0, 2.3)
Environmental					
- Any unusual climatic events during the first 35 DOC (or until the first signs of AHPND)	1.9	0.31	3.84	0.000	(1.4, 2.6)
Postlarval (PL) management					
- Source of PL (hatchery)					
- Small-sized hatchery			Reference		
- Medium-sized hatchery	1.6	0.59	1.20	0.230	(0.8, 3.3)
- Hatchery A	1.9	0.65	1.86	0.063	(1.0, 3.7)
- Hatchery B	1.0	0.30	0.03	0.979	(0.6, 1.8)
- Hatchery C	0.9	0.25	- 0.34	0.733	(0.5, 1.6)
- Hatchery D	1.8	0.50	2.02	0.044	(1.0, 3.1)
District					
DD			Reference		
HB	0.5	0.11	-3.14	0.002	(0.3, 0.8)
TD	5.3	2.20	4.05	0.000	(2.8, 12.0)
VC	14.4	5.74	6.71	0.000	(6.6, 31.5)
Constant	1.0	0.33	0.02	0.982	(0.5, 1.9)

Note: OR = odds ratio, SE = standard error, Z = standard normal deviate, 95 % CI = 95 % confidence interval

Multivariable logistic regression model

Water quality data could not be included in the multivariable logistic regression analysis due to a lot of missing data, ranging from 58.0–99.9 % of records. The pond depth factor was transformed into categorized variables with cut-off at ≤120 cm. and 120 cm. prior to being included in the regression model. The final multivariable logistic regression model of factors associated with AHPND cases in shrimp ponds in the Mekong Delta area of Viet Nam after controlling for the confounding effect of districts and farms is presented in Table 6. The prevalence of AHPND varied among the districts, with the highest prevalence in Vinh Chu District. The factors related to increased risk of AHPND occurring were the following: ponds with water depth equal to 1.2 m or below, ponds which experienced abnormal weather events during the first 35 DOC or until the first signs of AHPND, and ponds that used fertilizers and probiotics for water treatment.

Moreover, ponds that were supplied with PL from a specific hatchery (i.e. hatchery D) were more likely to have outbreaks of AHPND than ponds supplied from the other hatcheries. On the other hand, ponds that were supplied with minerals and algaecides for water treatment were shown have a reduced risk of AHPND occurring. No significant, biologically plausible interactions were detected.

Discussion

Farm characteristics and farm management

Every hectare increase in culture area of the farm increased the risk of AHPND occurrence by 1.32 times. The larger culture area might relate to more difficulty in pond management and reduced cost-effectiveness of using disinfectants to prevent the entry of the pathogen into the farm. This finding was similar to that of Corsin (2012) during an investigation of an unknown disease outbreak (now known later as AHPND) in Viet Nam in 2012. Farms being located close to other farms using the same AHPND-infected water source increased the risk by 5.07 times, and this was the most important farm-level risk factor. The same finding was reported in a similar study investigating an outbreak of AHPND in eastern Thailand (Kasornchandra et al. 2014; Boonyawiwat et al. 2017).

These findings indicate that the transmission of AHPND occurs via water. Pond preparation through sediment drying was associated with an increased risk of AHPND when compared to other pond preparation methods (such as flushing, ploughing or soil removal). This finding differed from the results of the study by Corsin (2012), also in Viet Nam, which the first time of his study indicated that washing the pond during pond preparation increased the risk of AHPND occurring. During the AHPND outbreak, shrimp farmers reduced their economic losses by delaying their crop operation. Leaving the pond empty and disinfecting the pond bottom by exposure to sunlight are some of the most cost-effective and convenient measures in this situation. About 92.7 % of study farms used these methods for cleaning their ponds. Such methods might be good for degradation of remaining organic matter in pond soil, but the depth of the degradation process is limited by the level of oxygen in the soil.

Usually, a high degree of organic matter degradation occurs only to a depth of 10–15 cm below the bottom surface, depending on type of soil. The organic matter under that level may remain and be a problem after adding water or during the crop operation. Sun-drying of the pond bottom may have eliminated all the microbes (Austin and Austin 1999) but this method created unequilibium of microbial community and a consequent lack of competition. After filling pond with water the fast-growing bacteria (such as many pathogenic *Vibrio* spp.) will have a good opportunity to have access to the rich nutrients and growth without competing with other microbes (Lavilla-Pitogo et al. 1998; Schryver et al. 2014). Therefore, the *Vibrio* spp. will dominate and recolonizing the environment. (Attramadal et al. 2015).

Pond management

Ponds with a water depth of 1.2 m. or below have a higher risk of AHPND due to the rapid change of water quality (e.g. water temperature, salinity, alkalinity, etc.), especially during periods of abnormal weather events. However, their interaction effect was not found to be significant, although a higher proportion of AHPND cases during unusual weather occurred in ponds with low water level (Fig. 2).

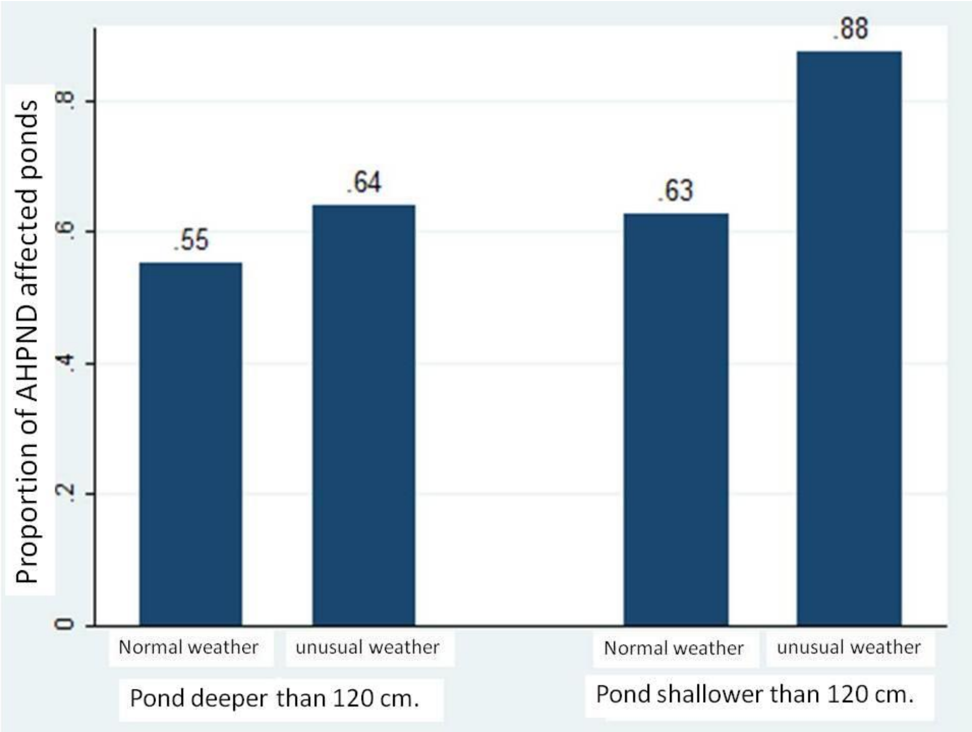

Fig. 2. Proportions of AHPND cases to total assessed ponds for shrimp ponds when unusual weather occurred with different pond water level.

Postlarval (PL) shrimp

Previous risk factors studies conducted in Viet Nam (Corsin 2012) and Thailand (Boonyawiwat et al. 2017) have found that source of PL is associated with an increased risk of AHPND outbreak at the pond level. Such findings suggest that the occurrence of AHPND in grow-out ponds may be related to stocking of infected PL (particularly PL infected with *Vibrio parahaemolyticus)* from hatchery and nursery facilities. Bacteria could be introduced to the facilities via various pathways, for example, through infected nauplius, contaminated water or feed, or water pipelines contaminated with bacteria. Moreover, bacteria could rapidly multiply when inappropriate management conditions occur in hatchery operations (i.e. high PL density, high organic load and high temperature). Disinfection water prior to use is a common practice in this stage of shrimp culture, but it lead to increase nutrient availability, reduced microbial number, lack of competition and predation induced the recolonization by heterotrophic bacteria (*r*-strategists) (MacArthur and Wilson 1967; Hess-Erga et al. 2010). Therefore, the numbers of bacteria in the water have been noted to increase after a few days post-treatment. Such bacterial loads may result from the growth of bacteria present in the water pipelines.

As a survival mechanism, bacteria belonging to the family Vibrionaciae can form biofilms when in a harmful environment, such as when in contact with a disinfectant (Gode-Potratz and McCarter 2011). The increase in the number of bacteria in hatchery and nursery facilities makes it possible for PL to become infected through these contaminated sources.

Water management

Water management through fertilization to promote phytoplankton growth is a common process in grow-out farms. However, the use of organic fertilizers (e.g. chicken manure) may promote the growth of harmful bacteria in the shrimp pond. In addition, applying a large amount of fertilizer to the pond may promote an increase in phytoplankton, increased water pH, a wide range of daily pH fluctuation, and high nitrogen content. *Vibrio parahaemolyticus* is an enteric bacterium that prefers high pH and high levels of nitrogen. Adding an algaecide may reduce phytoplankton numbers, changing the water to a suboptimal condition for *Vibrio* spp. Shrimp need to absorb minerals (i.e. calcium, magnesium and potassium) from water to meet their requirements for growth. Thus adding minerals to the pond is another way to support their health status and promote normal growth performance.

This study also found that the use of probiotics is another risk factor for AHPND outbreak. This is in agreement with the study done by Corsin (2012), but did not support suggestions that why the application of probiotics has a positive impact on the survival of shrimp grown in AHPND-affected areas (Panakorn 2012; Schryver et al. 2014). Probiotic technology can change microbial species composition in shrimp ponds by adding selected bacterial species that displace deleterious normal bacteria. While, probiotic bacteria may do well in terms of competition with ubiquitous or opportunistic bacteria. But the application of recent commercial strain of probiotics gave uncertain results with regard to Vp_{AHPND} infection. Therefore, the studies to find a specific bacterial strain that can effectively control Vp_{AHPND} bacteria were need, including the dose and frequency of application to prove its efficacy.

Measures to control disease spread have been widely implemented on shrimp farms in Thailand, including the improvement of on-farm and shrimp health management practices. However, the effectiveness of applying probiotics, traditional herbal medicines and molasses has not been evaluated (FAO 2013b). Simultaneously, a number of research collaborations have initiated to control the occurrence of AHPND on shrimp farms and to identify risk factors associated with AHPND cases (FAO 2013).

A preliminary study of AHPND conducted in eastern Thailand (Kasornchandra 2014) has suggested that the source of PL shrimp, the total amount of feed used before the disease event, and water disinfection with chlorine are associated with AHPND occurrence on shrimp farms. While the findings have initiated discussion around farm management practices, there has yet to be a comprehensive study to identify management practices that may affect the risk of AHPND on shrimp farms. A better understanding of farm management practices related to disease would inform AHPND policies on shrimp farms, control strategies, and risk management plans.

Conflict of interest

There were no known conflicts of interest**.**

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