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A cohort study of 196 Thai shrimp ponds reveals a complex etiology for early mortality syndrome (EMS)

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ABSTRACT

Early mortality syndrome (EMS) refers to acute mortality within approximately 35 days of shrimp culture. It is often equated with acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio* isolates that produce Pir-like toxins. To better understand EMS, 200 Thai shrimp ponds were randomly selected prior to stocking and subsequently sampled from 19/08/2013 to 23/04/2014 to determine the pond prevalence of various pathologies, and particularly those targeting the shrimp hepatopancreas (HP). Ponds exhibiting acute mortality within 35 days were labeled EMS ponds while others were labeled non-EMS ponds. Shrimp samples from each pond (10) were subjected to histological analysis focused on hepatopancreatic (HP) lesions. Similar samples (10) were tested by PCR for the microsporidian *Enterocytozoon hepatopenaei* (EHP), white spot syndrome virus (WSSV), yellow head virus (YHV) and *Vibrio* that cause AHPND. Preservation failed for 4 ponds. Results from the 196 ponds remaining were divided, into 4 groups based largely on HP histology: (1) ponds positive for AHPND by histology and/or PCR at 20.9% prevalence (41/196); (2) ponds showing HP bacterial lesions at 14.8% prevalence (29/196); (3) ponds showing collapsed HP tubule epithelia at 25.0% prevalence (49/196); and (4) ponds showing no HP pathology (normal HP) at 39.3% prevalence (77/196) ponds. The overall prevalence of EMS ponds (i.e., mortality ≤35 days) was 16.3% (32/196), but only 18/32 (56.3%) of these fell within AHPND Group 1. The other 14 EMS ponds (43.7%) were divided among the remaining three groups: Group 2 with HP bacterial lesions (3/32 = 9.4%), Group 3 with collapsed HP tubule epithelia (1/32 = 3.1%) and Group 4 with normal HP histology (a surprising 10/32 = 31.2%). Across all groups, prevalence of ponds exhibiting aggregated transformed microvilli (ATM) was high at 79.8%, as was prevalence of EHP at 60.7%. The overall prevalence of WSSV was low at 4.1% (8/196), while YHV was not found. Overall, the results indicated that equating EMS with AHPND without confirmatory analysis could lead to overestimation of its impact on shrimp aquaculture and to failure in recognizing other important causes of early mortality.

1. Introduction

Early mortality syndrome (EMS) refers to acute mortality within approximately 35 days of stocking shrimp ponds together with gross signs of a dark and shrunken hepatopancreas, and it is often mistakenly equated to acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio parahaemolyticus* isolates that carry a unique pVA plasmid encoding the Pir-like toxins Pir^{vp}A and Pir^{vp}B (Han et al., 2017; Kondo et al., 2015; Thitamadee et al., 2016; Tran et al., 2013; Xiao et al., 2017). EMS caused high production losses beginning in China in 2009

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followed by spread to other countries in Asia and eventually to Mexico (Thitamadee et al., 2016). It has been proposed that the spread in Asia was probably aided by the widespread transboundary trading of living animals such as polychaetes and clams as shrimp broodstock feeds. This proposal is supported by PCR positive test results for AHPND bacteria in polychaetes and by the fact that Asian countries that restrict such imports experienced delayed entry of AHPND (e.g., the Philippines) (de la Peña et al., 2015) or remained free of AHPND (e.g., Indonesia).

To better understand EMS and AHPND, to determine risk factors associated with them and to determine their prevalence in Thailand, two epidemiological study projects have been carried out. The first was a retrospective, observational, case-control study (Boonyawiwat, et al., 2017) of shrimp farms covering the period of August 2013 to April 2014. The second was a cohort study carried out from 19/08/2013 to 23/04/2014 using 200 shrimp ponds selected prior to stocking in 7 Thai provinces where EMS disease outbreaks had previously been reported. This report concerns results from a sub-project on disease diagnosis for the 200 ponds in the second (cohort) study above. It covers only histological analysis and PCR tests of specimens to determine the prevalence of various pathologies (particularly those of the hepatopancreas) in the study ponds. Briefly, 20 shrimp specimens were taken from each pond in the study, 10 for histological analysis and 10 for PCR analysis focusing mostly on pathogens targeting the shrimp hepatopancreas (HP). However, white spot syndrome virus (WSSV) and yellow head virus type-1 (YHV-1) were also included even though they target tissues of ectodermal and mesodermal origin (i.e., not endodermal, tubule epithelial cells of the HP). They are important because they sometimes cause early mortality in shrimp ponds and might, in the absence of confirmatory tests, be included by farmers under the umbrella of EMS outbreak ponds. The overall objective was to determine whether there was a common pathology such as AHPND in ponds designated as EMS ponds.

This is not an epidemiological study but a simple pond prevalence analysis of various shrimp pathologies in randomly selected ponds in Thailand during a specified time interval. It does not include analysis of the correlation between pathogen prevalence and production information or disease risks factors for the study ponds. However, it does include analysis of positive and negative correlations among the pathologies and it clearly reveals that the designation EMS is misleading and cannot be equated with any particular pathology or pathogen. At the same time, the prevalence results described herein will be included as a data subset in a subsequent, independent report on a multivariate epidemiological analysis of the risk factors and outcomes associated with EMS, AHPND and other pathologies described in this report.

2. Materials and methods

2.1. Shrimp samples

Shrimp samples used for analysis in this study were collected in an overall project involving 133 shrimp farms randomly selected in advance of stocking from 7 provinces in Thailand where major shrimp cultivation occurs and where outbreaks of EMS/AHPND had previously been reported (Fig. 1). Samples were collected from 19/08/2013 to 23/04/2014 from a total of 200 ponds (average of 1.5 sample ponds per farm) (Table 1). Sample ponds were selected by using a two-step sampling method. The first step was to select farms using the probability proportional to size (PPS) sampling method with the list of farms registered with the Thai Department of Fisheries. In cases where selected farms had terminated their culture activities before the sampling period or did not agree to be involved in the project, new farms in the same area were selected to fulfill the required number of farms. At the second stage of sampling, if there were > 3 shrimp ponds on a farm, the ponds were selected according to the simple random sampling (SRS) method. If there were < 4 culture ponds in the farm, all of the ponds were selected for the study.

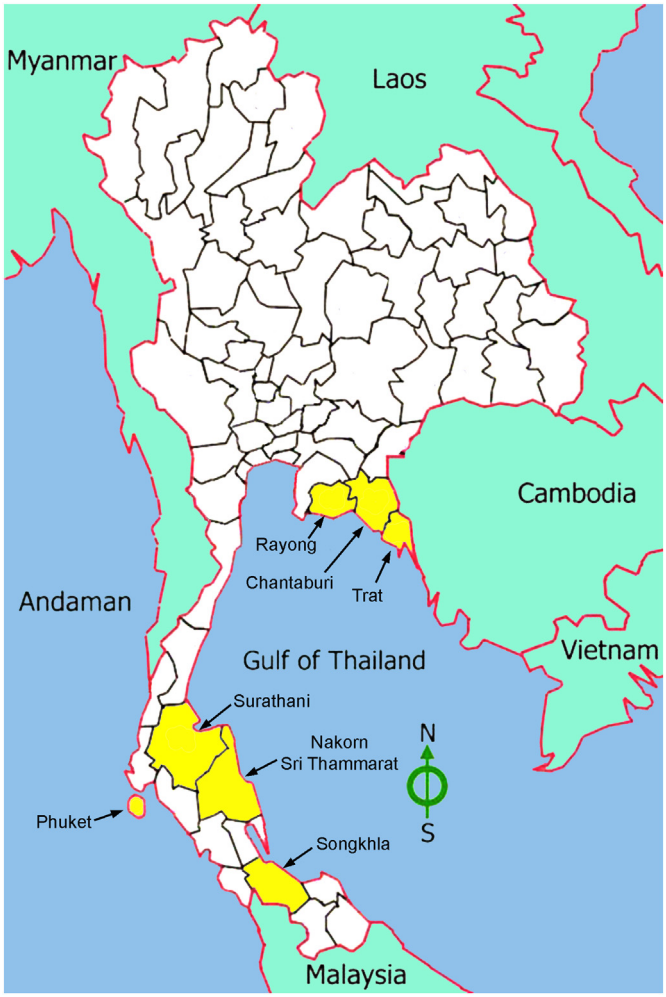

Fig. 1. Map of Thailand showing the provinces of major shrimp cultivation where the sampling sites for this study were located.

Table 1
Breakdown of the number of ponds sampled per farm in this study of 200 sample ponds from 133 farms (an average of 1.5 ponds per farm).

#Ponds sampled	#Farms	% of Farms	#Ponds	% of Ponds
1	101	75.9	101	50.5
2	15	11.3	30	15.0
3	7	5.3	21	10.5
4	6	4.5	24	12.0
5	1	0.8	5	2.5
6	2	1.5	12	6
7	1	0.8	7	3.5
Totals	133	100	200	100

The primary purpose or our sub-project was to provide pathological and prevalence information that could serve as a data subset for a subsequent independent epidemiological analysis of risk factors associated with AHPND/EMS outbreaks that will be reported elsewhere. For the purposes of the overall project, the case definition for EMS was the occurrence of unusual mortality within the first 35 days (≤35 days) after stocking of shrimp ponds with post larvae and accompanied by gross signs related to EMS (Tran et al., 2013). As stated in the introduction the work described herein covers only histological analysis and PCR tests of specimens to determine the prevalence of various pathologies (particularly those of the hepatopancreas) in the study ponds. Shrimp in these ponds were collected as soon as possible at the time of an EMS outbreak. Shrimp in ponds that did not show these signs

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