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## Infectious Myonecrosis Virus (IMNV) in Pacific White Shrimp (*Litopenaeus vannamei*) in Indonesia

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Key words: disease, infectious myonecrosis virus (IMN), Pacific white shrimp, *Litopenaeus vannamei*, Indonesia

### Abstract

Penaeid shrimp culture has become a leading export fishery in Indonesia. The Pacific white shrimp (*Litopenaeus vannamei*) was unofficially introduced to Indonesia in 1999, and received government approval in 2001. By the end of 2007, the Pacific white shrimp was cultured in over 17 provinces. The main constraints of shrimp culture have always been diseases, especially those caused by viral agents. Taura syndrome (TS) disease was detected in Indonesia in 2002 and the disease currently affects at least ten provinces. Infectious myonecrosis (IMN) is an emerging *L. vannamei* disease, first detected in Indonesia in May-June 2006. IMN disease causes significant mortality in growout ponds and is characterized by acute onset of gross signs: focal to extensive whitish necrotic areas in the striated muscle, especially of the distal abdominal segments and the tail fan. The white necrotic areas redden, similar to the color of cooked shrimp. The outbreak results in elevated mortality that was initially associated with a chronic course of persistent low level mortality. To date, IMN has been detected in East Java, Bali, and West Nusa Tenggara provinces. This paper reviews studies of IMN disease of Pacific white shrimp in Indonesia: outbreaks, surveillance, diagnosis, and control measures.

### Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, was initially introduced in Indonesia in 1999 as an alternative species for aquaculture besides the local shrimp species, *Penaeus monodon* and *P. merguensis*. The Indonesian government allowed import of Pacific white shrimp on 10 October 2000 for research purposes only. The exotic shrimp was imported from Taiwan and Hawaii. On the basis of Ministerial Decree No. 4/2001 dated 14 July 2001, import of Pacific white shrimp was allowed for culture purposes. To protect sustainability of the Pacific white shrimp industry in the country, only good quality virus-free broodstock are approved for import. By the

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end of 2007, *L. vannamei* were being cultured in at least 17 provinces (East Java, Central Java, West Java, Yogyakarta, Banten, Bali, West Nusa Tenggara, Lampung, South Sumatra, Riau, Bengkulu, West Sumatra, North Sumatra, South Sulawesi, South Kalimantan, East Kalimantan, and West Kalimantan).

Since 2002, the main constraint encountered in Pacific white shrimp culture in Indonesia has been associated with disease outbreaks, especially those caused by viral agents. In early 2003, a serious outbreak occurred in northern East Java. This was the first such outbreak in Indonesia and the causative agent of the disease was taura syndrome virus (TSV). The disease was most likely brought to the country by illegal import of *L. vannamei* from sources of unreliable health status. Since then, TSV disease has spread and by 2007 was detected in at least 10 provinces. In mid 2006, there were anecdotal reports of disease outbreaks in Pacific white shrimp in Situbondo district. Gross signs of white muscle were similar to infectious myonecrosis virus (IMNV) outbreaks in Brazil (Senapin et al., 2007). The Indonesian IMNV sample had 99.6% nucleic acid sequence identity (29 differences in 7.5 kb) to that of Brazilian IMNV reported at GenBank (Senapin et al., 2007).

At present, four significant viral diseases have been reported in cultured Pacific white shrimp in Indonesia: TSV, IMNV, white spot syndrome virus (WSSV), and infectious hypodermal and hematopoietic necrosis virus (IHHNV). This report surveys epidemiological data on the outbreak, disease diagnosis, and control measures of IMN in Pacific white shrimp culture in Indonesia.

### Materials and Methods

**Surveillance.** The IMNV survey was conducted in 2006 and 2007. The survey focused on high risk areas of East Java and the nearby provinces of Bali and West Nusa Tenggara. Sampling sites were identified based on development and intensity of Pacific white shrimp culture. The surveillance design was developed according to the Survey Toolbox for Aquatic Animal Diseases (Cameron, 2002).

Shrimp farms were identified through stratified random sampling based on the latest district records of the Fisheries Service Agency and sampled randomly at defined spot areas. In addition, information was collected from respondents on semi-structured questionnaires. Data collection and other information mainly focused on IMNV outbreaks and control measures.

**Sampling and diagnostic techniques.** Shrimp samples were taken from hatcheries and growout ponds in three spot areas in each district, representing a sampling site. Samples consisted of 100-150 postlarvae or 30 adults. Postlarvae from the same population were pooled and preserved in 80% alcohol mixed with 20% glycerol in the same bottle. Adult shrimp with obvious clinical signs (Poulos and Lightner, 2006) were taken from IMN-suspected ponds and preserved individually in 80% alcohol mixed with 20% glycerol.

IMNV was diagnosed on three levels. Level I included behavioral and clinical signs and mortality rates. Level II focused on the histopathology of infected shrimp. Level III involved analysis by polymerase chain reaction (PCR) using IMNV IQ2000 Detection and Prevention System (Farming Intelligene Tech. Corp.). Muscle tissue from the sixth abdominal segment or gill was homogenized in TRIzol reagent (Invitrogen) and RNA was extracted according to the manufacturer's instructions. Pooled samples of 5-10 adult shrimp constituted one IMNV test. When the result was positive, individual shrimp were further analyzed. When the result was negative, no further tests were conducted.

### Results

**Surveillance.** The IMN virus spread rapidly. It was restricted to the Kapongan municipality in Situbondo district, East Java, in May-June 2006 (Fig. 1). In July-August 2006, outbreaks of a disease with similar clinical signs were reported from shrimp farms near the site where the first IMN outbreak occurred. Since then, IMN outbreaks spread to shrimp ponds in neighboring areas. By

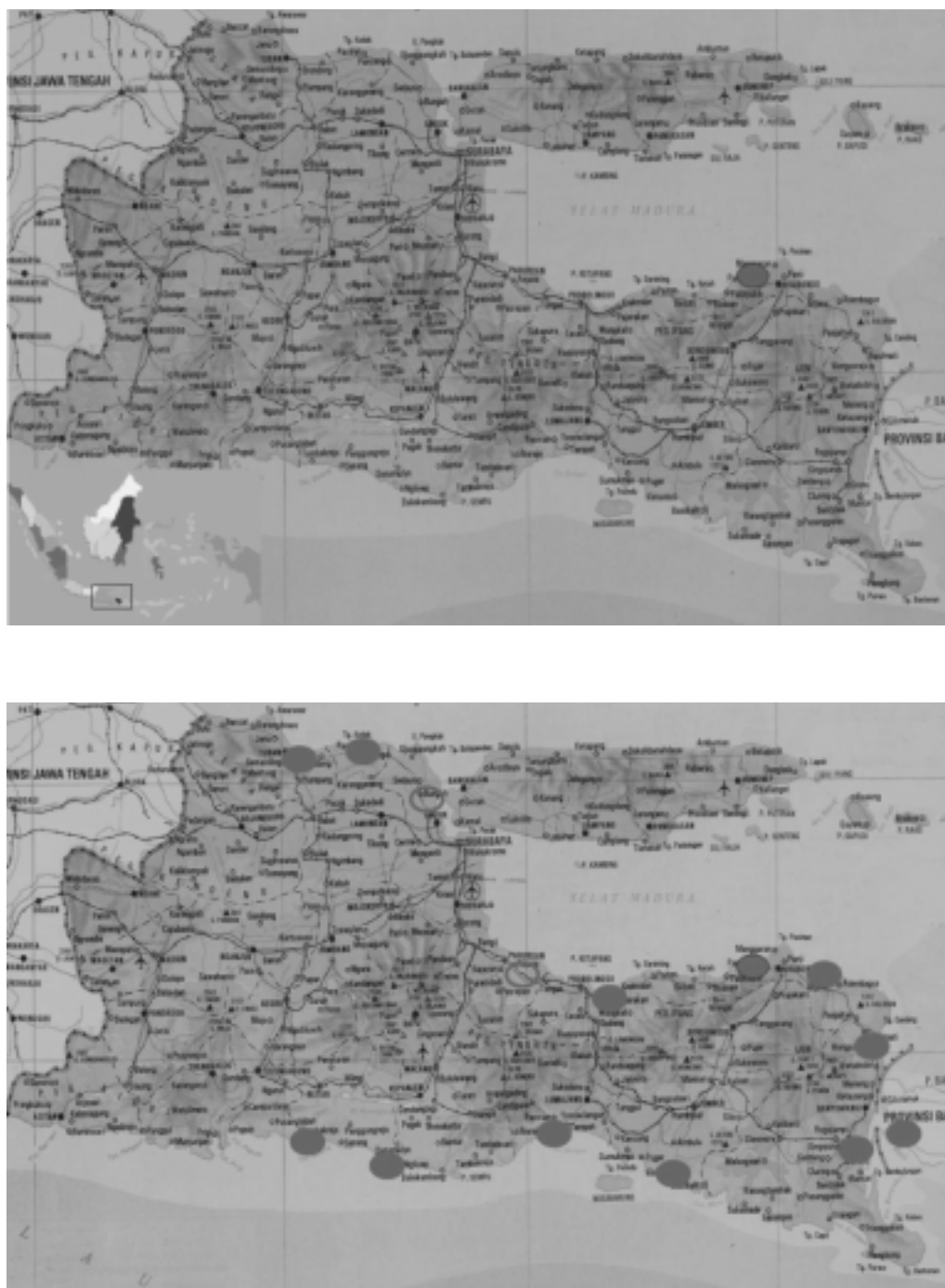


Fig. 1. Distribution of infectious myonecrosis virus (IMNV) in East Java and Bali (a) in July-August 2006 and (b) by the end of 2007 (clear circles = IMNV-negative; solid circles = IMNV-positive).

the end of 2007, the virus was detected in shrimp production centers in East Java, western Bali, and West Nusa Tenggara.

The outbreak in Kapongan municipality occurred in shrimp that had been in ponds for over 70 days. Mortality ranged 10-30%. The postlarvae used to stock the ponds originated in a hatchery in Situbondo, a district well known as a center of penaeid shrimp hatcheries in Indonesia. Information on IMN outbreaks in three shrimp farms in Situbondo district is summarized in Table 1.

IMN outbreaks caused significant mortalities in juvenile and subadult pond-reared stocks. In 2006, they occurred mainly in shrimp that had been in the ponds 70 days but, recently, outbreaks have occurred as early as 30 days in the pond. They usually occur in growout ponds at 30-90 days of culture and do not depend on the season. Mortality due to IMN is 10-30%, but if shrimp are also infected by TSV the mortality rate can exceed 40%.

According to farmers, IMN outbreaks occurred mainly in shrimp farms without reservoir ponds. Occasionally, IMN outbreaks occurred in farms with reservoir ponds. In IMN-endemic areas, the disease usually occurred earlier in shrimp farms without reservoirs.

**Level I diagnosis.** As the outbreak continued, various clinical signs were observed or reported. General indicators of IMNV infection in Pacific white shrimp include lethargy, loss of balance, swimming on the water surface during day time, abrupt drop in feeding rate, whitish necrotic areas in striated muscles that become reddened similar to cooked shrimp, and elevated mortality. These clinical signs may suddenly appear following stress, e.g., due to a change in temperature or salinity. Severely-affected shrimp that were feeding just before the onset of stress and have a full gut may become moribund. Mortality can be instantaneously high and continue for several days. Generally, the disease outbreak is indicated by a rise in the mortality rate and progresses to a more chronic course accompanied by persistent low level mortality. The only consistent and pathognomonic clinical sign of the disease is focal to extensive whitish necrotic areas in the striated muscles, especially of the distal abdominal segments and tail fan, that become reddened, similar to the color of cooked shrimp (Poulos and Lightner, 2006). Therefore, these clinical signs were used for the presumptive diagnosis of IMN disease (Fig. 2).

Table 1. Taura syndrome virus (TSV) and infectious myonecrosis virus (IMNV) in Pacific white shrimp (*Litopenaeus vannamei*) in three shrimp farms in Situbondo in 2007.

Farm	Period	No. of ponds	Postlarvae from local domesticated broodstock				Postlarvae from imported specific pathogen free (SPF) broodstock			
			No. of ponds	Infected with TSV	Days after stocking	IMNV	No. of ponds	Infected with TSV	Days after stocking	IMNV
1	Jun-Jul	18	10	1	40	7	8	3	30	1
2	Aug-Sept	26	15	2	40	9	11	4	30	2
3	Oct-Nov	20	12	1	30	4	8	1	30	-
					60					80
					75					90
					60					-

*Level II diagnosis.* Acutely infected shrimp present lesions with coagulative muscle necrosis, often with edema. In shrimp recovering from acute disease or in the more chronic phase of the disease, myonecrosis appears to progress from coagulative to liquefactive necrosis. The progression is accompanied by hemocytic infiltration and fibrosis. Typical histological changes due to IMNV infection are shown in Fig. 3.

*Level III diagnosis.* PCR of shrimp collected during the first outbreak in Situbondo district (2006) showed that the intensity of the viral infection ranged from light to moderate according to the IMNV IQ2000 interpretation chart (Fig. 4). Subsequent analysis revealed that the Indonesian IMNV had 99.6% nucleic acid sequence identity to that of the Brazilian IMNV reported at the GenBank (GenBank accession No. EF061744). Although samples of postlarvae collected in 2006-2007 were IMNV-negative, when reared in growout ponds, they tested IMNV-positive by PCR. A similar phenomenon was observed in all subsequent crops during the dry and rainy sea-

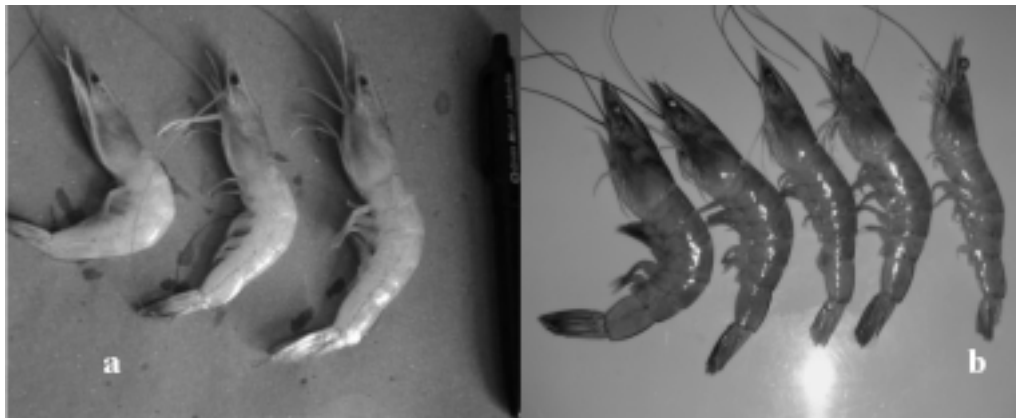


Fig. 2. Clinical signs of Pacific white shrimp infected by infectious myonecrosis virus (IMNV): (a) focal to extensive whitish necrotic areas in the striated muscle and (b) reddened necrotic areas, similar to the color of cooked shrimp.

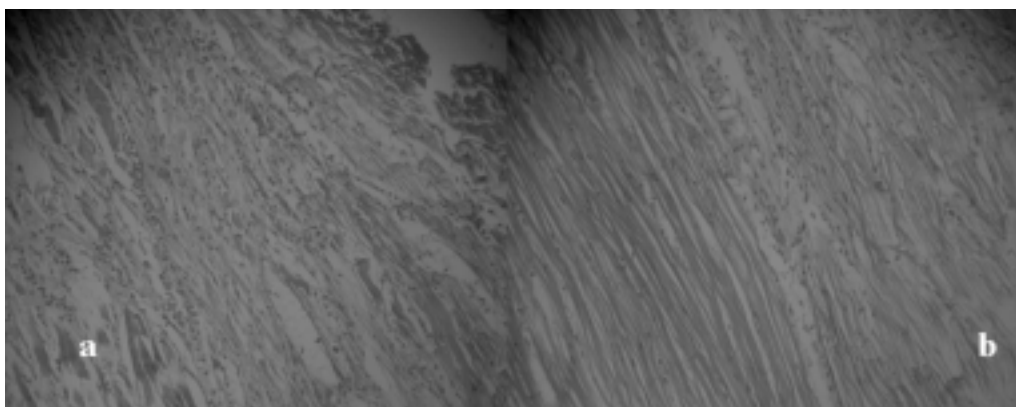


Fig. 3. Histological changes in Pacific white shrimp infected by infectious myonecrosis virus (IMNV): (a) myonecrosis, accompanied by hemocytic infiltration and fibrosis and (b) normal skeletal muscle, in lower left corner (hematoxylin/eosin stain; magnification 100x).

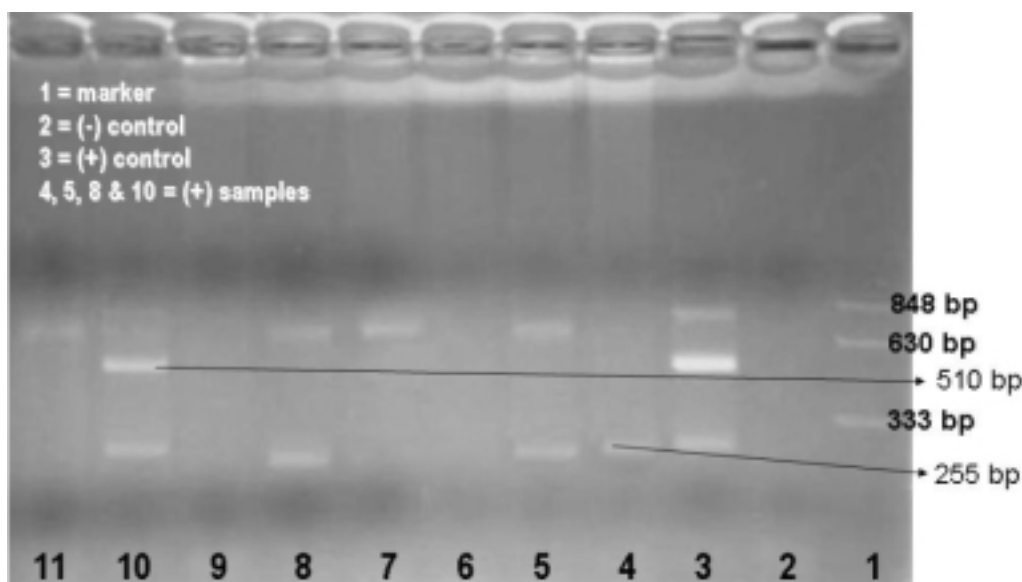


Fig. 4. Polymerase chain reaction (PCR) of Pacific white shrimp collected during the first outbreak of infectious myonecrosis (IMN) disease in May-June 2006 in Indonesia.

sons. All samples collected in July-September 2007 were lightly infected by IMNV. The PCR profile of diseased shrimp from the major infected areas of Probolinggo and Banyuwangi is shown in Fig. 5.

**Control measures.** As soon as IMV infection is confirmed on a farm, it is usually quarantined and infected ponds are disinfected to prevent contamination of other ponds. In the early stage of infection, when mortality rates are still low, routine control measures include: (a) stabilizing water quality parameters, especially temperature, salinity, and pH, (b) increasing aeration as much as possible, (c) applying feed additives (microencapsulated ascorbic acid), (d) applying sugar molasses (25% of feeding rate/day) or a probiotic, and (e) decreasing or stopping feeding for a while.

### Discussion

An IMN outbreak in Indonesia was first recorded in Situbondo district, East Java. Situbondo and Banyuwangi are endemic for IMNV and also the country's largest producers of penaeid shrimp larvae/postlarvae (including Pacific white shrimp). The geographical distribution of IMNV in Indonesia spread faster than its control program. Therefore, the disease has serious potential of spreading in the country. The main mechanism of disease transmission is still unclear. However, it is strongly suspected that the disease is transmitted mainly through shrimp broodstock and postlarvae.

It is unclear how the disease originally came into Indonesia. Broodstock or postlarvae from Brazil may have been smuggled into the country for use in a commercial hatchery (Senapin et al., 2007). If so, it may explain the close identity of the genome sequences of IMNV from Indonesia and IMNV from Brazil and may constitute another example of the unfortunate transfer of shrimp pathogens over large geographical distances by careless movement of contaminated stocks for aquaculture. Many viral pathogens are restricted in their geographical distribution and

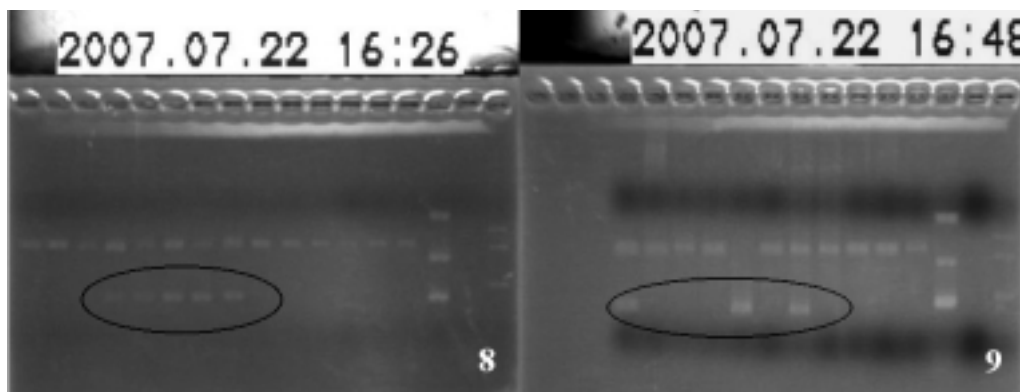


Fig. 5. Polymerase chain reaction (PCR) of Pacific white shrimp infected by infectious myonecrosis virus (IMNV), collected from (a) Probolinggo and (b) Banyuwangi.

their spread to new areas can have disastrous consequences (Flegel, 2006). Unregulated trans-boundary movement of shrimp broodstock, postlarvae, and products is the major cause of the spread of pathogens between regions.

Recently, IMNV was detected in Pacific white shrimp farms in South Kalimantan (Supriyadi, pers. comm.). The seed used in these farms originated from Situbondo (East Java) and infected postlarvae/broodstock and contaminated shrimp ponds/water were the most likely sources of the infection. Risk factors such as water quality and feed management may play a role in disease development. Outbreak of the disease was strongly associated with algal bloom, extreme fluctuations of salinity, pH, or temperature, and, possibly, low quality feeds (Poulos et al., 2006). In contrast to postlarvae produced from domesticated local broodstock, postlarvae produced from imported specific pathogen free (SPF) broodstock seemed to be more resistant to IMNV infection but more sensitive to TSV infection. Unfortunately, both TSV and IMNV were always detected in all districts of East Java.

Most shrimp farmers prefer to use behavior, clinical signs, mortality rate, and PCR to detect the presence of IMNV infection. These are the most practical and popular methods for diagnosing the disease. Histopathology is time consuming and requires a well-trained shrimp pathologist, thus, it is used mainly for confirmatory diagnosis. Unstable PCR results during the 2006 and 2007 IMNV outbreaks were attributed to factors that can affect the sensitivity and specificity of the diagnostic kit. As detection of IMNV by PCR assay is still new in Indonesia, a standardized and harmonized PCR application is urgently needed to ensure high quality results.

Increased risk of introduction and distribution of pathogens accompanies increased traffic throughout the country of Pacific white shrimp broodstock, postlarvae, and shrimp products. Since there is no effective treatment for IMNV infection, shrimp farmers can protect their farms and shrimp only by preventing the disease. Recommended disease prevention strategies include the use of SPF seed, screening of seed, waterborne crustaceans, and plankton, and examining the relationship between viral load and clinical signs to determine when to harvest. Implementation of Ministerial Decree No. 17/2006 will help create IMNV-free areas in the future. This decree was issued to protect the country from the introduction of exotic diseases and to prevent the spread of IMNV from infected zones to uninfected zones.

In cases of acute onset of gross signs, elevated mortality, and persistent low level mortality, shrimp of marketable size should be harvested immediately. The pond and its contents (water, aeration system, feeding trays, etc.) should be disinfected by applying chlorine at  $\geq 30$  ppm for



several days. Once the virus has been eradicated from a site, reintroduction should be prevented. Since the source of infection is usually infected shrimp or postlarvae, or contaminated water and equipment, postlarvae and broodstock should be obtained from reliable IMNV-free sources. Potential carriers must be rigorously excluded from farms in endemic areas, and water and equipment which may have been used at infected sites must be disinfected.

It is suspected that IMNV can be transmitted horizontally and vertically. Thus, the virus can be transmitted from broodstock to nauplii, from live feed or other animals, via cannibalism or by sharing tools, etc. When a virus is carried into the culture environment, all potential carriers are to be screened and those infected with the disease should be discarded. To prevent vertical transmission, all broodstocks must be screened; to prevent horizontal transmission, virus-free postlarvae must be stocked and good management practices followed. The key to successful prevention is quick action.

The use of SPF Pacific white shrimp is an important step but the fact that non-SPF *L. vannamei* are still being used in the country can cause health problems. Transboundary movement of broodstock still plays a major role in the spread of shrimp pathogens. The Indonesian government strongly recommends that import of Pacific white shrimp for culture be brought only from Hawaii and Florida (USA). Also, the practice of holding broodstock of different species in the same holding space by brokers should be avoided.

We have no accurate data on economic losses resulting from IMN disease in Pacific white shrimp in Indonesia. However, many farmers reported lost profits due to the disease. Concerns include the need for government support of disease control programs, including restrictions on the movement of live shrimp from infected to uninfected zones. IMN has been added to the list of diseases under the National Fish Quarantine through Ministerial Decree No. 17/2006. It is hoped that awareness of this newly emerging pathogen will motivate shrimp farmers and government authorities to heighten measures against its further spread.

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