Vol. 50: 1–8, 2002 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published June 21

Usefulness of dead shrimp specimens in studying the epidemiology of white spot syndrome virus (WSSV) and chronic bacterial infection

C. V. Mohan[1,*], F. Corsin[2], P. C. Thakur[1], P. A. Padiyar[1], M. Madhusudan[1], J. F. Turnbull[3], N. V. Hao[4], K. L. Morgan[2]

[1]Fish Pathology Laboratory, Department of Aquaculture, College of Fisheries, University of Agricultural Sciences, Mangalore 575002, India

[2]Department of Veterinary Clinical Science and Animal Husbandry, The University of Liverpool, Leahurst, Chester High Road, Neston CH64 7TE, United Kingdom

[3]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom

[4]Research Institute for Aquaculture N. 2, 116 Nguyen Dinh Chieu Street, Ho Chi Minh City, Vietnam

ABSTRACT: This paper describes the utility of dead shrimp samples in epidemiological investigations of the white spot syndrome virus (WSSV) and chronic bacterial infections. A longitudinal observational study was undertaken in shrimp farms in Kundapur, Karnataka, India, from September 1999 to April 2000 to identify risk factors associated with outbreaks of white spot disease (WSD) in cultured *Penaeus monodon*. As a part of the larger study, farmers were trained to collect and preserve dead and moribund shrimp (when observed) during the production cycle. At the end of the production cycle, 73 samples from 50 ponds had been collected for histopathology and 55 samples from 44 ponds for PCR. Intranuclear viral inclusion bodies diagnostic of WSSV infection were detected in dead samples from 32 ponds (64%). Samples of dead shrimp from 18 ponds (36%) showed no histopathological evidence of WSSV infection. However, of these, samples from 13 ponds (26%) showed clear evidence of shell, oral, enteric and systemic chronic inflammatory lesions (CIL) in the form of haemocytic nodules, typical of bacterial infection. Samples from 5 ponds (10%) were negative for both WSSV and CIL. Samples from 8 ponds had dual WSSV and CIL, although both WSSV and CIL were only observed in the same shrimp from 1 pond. Useful information was obtained from these shrimp despite the presence of post-mortem changes. Samples from 19 ponds (43%) tested positive for WSSV by 1-step PCR and samples from an additional 10 ponds (22.7%) were positive by 2-step nested PCR. Samples from 15 ponds (34.1%) were negative for WSSV by 2-step nested PCR. There was moderate to substantial agreement between PCR and histopathology in the diagnosis of WSSV infection in dead shrimp. WSSV infection in dead shrimp was significantly associated with crop failures as defined by a shorter length of the production cycle (<90 d) and lower average weight at harvest (<22 g). WSSV infection was also associated with lower survival (<50%), but this was not significant. Ponds with CIL did not experience any crop failures, and the presence of CIL was significantly associated with successful crops. The study demonstrates that samples of dead shrimp can provide useful information for disease surveillance and epidemiological investigations of WSSV and chronic bacterial infections.

KEY WORDS: White spot syndrome virus · Chronic inflammatory lesion · Epidemiology · Dead shrimp · PCR · Histopathology

INTRODUCTION

White spot disease (WSD) caused by white spot syndrome virus (WSSV) was first detected in Japan and China in 1993 (Momoyama et al. 1994, Nakano et al. 1994, Zhan et al. 1998). Since then it has spread throughout Asia (Wongteerasupaya et al. 1995, Anonymous 1997, Mohan et al. 1998, Park et al. 1998) and has recently been reported in the Americas (Calderon et al. 2000). Factors including stocking of infected post-

*E-mail: cv_mohan@yahoo.com

larvae (Limsuwan 1997, Flegel & Alday Sanz 1998, Mushiake et al. 1999), the addition of contaminated water (Nakano et al. 1994, Chou et al. 1998), the presence of WSSV carriers (Lo et al. 1996a, Kanchanaphum et al. 1998, Maeda et al. 1998) and stress (Chou et al. 1995, Lo et al. 1996a, Sudha et al. 1998) have been suggested as possible risk factors for WSD outbreaks.

Samples of moribund shrimp collected from the field have been routinely and effectively used in shrimp disease diagnostics (Lightner 1996, Kasornchnadra et al. 1998, Mohan et al. 1998). Owing to the risk of post-mortem changes, it is not normal to use dead shrimp for any aquatic animal diagnostic work. However, as part of a larger epidemiological study, farmers were asked to record the occurrence of dead shrimp in their ponds during the production cycle on a daily basis and to collect and preserve samples of them.

This paper examines the utility of these dead shrimp samples in epidemiological investigations. The objectives were to assess farmer compliance, to assess whether samples of dead shrimp could provide any indication of the health status of the pond population, to determine the possibility of using dead shrimp to examine the cause of death, and to compare PCR and histopathological assay results in the samples. The potential for diagnoses based on samples of dead shrimp as a predictor for failure or success was also examined using length of production cycle, survival and average weight at harvest as criteria.

MATERIALS AND METHODS

A longitudinal observational study was undertaken in shrimp farms of Kundapur, Karnataka, India, from September 1999 to April 2000 in order to identify risk factors associated with outbreaks of WSD in cultured *Penaeus monodon*. One hundred ponds were randomly selected, of which 70 were enrolled. In brief, variables related to pond preparation, stocking, post-larvae (PL), water quality, pond management and harvest were collected directly by the research assistants and through questionnaires. Samples of PL at stocking (500), shrimp at 6 wk (100) and at harvest (400) were collected from each pond for PCR and histopathological investigations. In addition, samples of plankton, wild crustaceans and other animals in the ponds were collected for WSSV screening.

Sample collection. The research assistants visited the ponds on a regular weekly basis from stocking to harvest. The daily pond management details were recorded by the farmers in a pre-tested farmer recording sheet that also included details of the number of dead shrimp at the pond edge on a daily basis. Sample collection bottles were first provided to each of the farmers 3 wk post-stocking. The sample collection bottles included one 10 ml bottle with methanol and one 500 ml bottle with neutral buffered formalin for each farmer. Farmers were trained through discussions and pictorial instructions to fix 1 swimming leg (pleopod) per dead shrimp in methanol for PCR and the rest of the whole shrimp in 10% neutral buffered formalin for histopathology. The weekly recording sheets and samples of dead shrimp, if any, were collected by the research assistants during the weekly visits, and at the same time blank recording sheets and a new set of preservative-filled and labelled sampling bottles for the next week were provided.

Sample processing and diagnosis. The samples were processed for histopathology following standard procedures (Lightner 1996). Depending on the number of shrimp available in each sample, a minimum of 4 shrimp per sample was sectioned and examined for histopathological evidence of WSSV infection. When no WSSV intranuclear inclusions were observed in the sample, all the available shrimp in that sample were examined to confirm the absence of WSSV. All the pleopods were cut, pooled and homogenised and used for DNA extraction and subsequent PCR analyses. The alkaline method (i.e. homogenisation of the sample in 0.05N NaOH and 0.025% SDS (sodium dodecyl sulphate) and boiling for 10 min) was used for DNA extraction (Chanratchakool pers. comm.) and the samples were analysed by 2-step nested PCR according to Lo et al. (1996b).

Data analysis. A pond was considered positive for WSSV infection when 1 or more shrimp tested positive by histopathology and/or when 1 or more samples tested positive by 2-step nested PCR. The kappa statistic was used to assess the level of agreement between histopathology and PCR results. Criteria for interpreting kappa test results were taken from Thrushfield (1986). The 2-step nested PCR and histopathology results on a pond basis were examined for associations with the outcome variables. The outcome variables selected for the analyses were the length of production cycle, average weight and survival at harvest. The median length of production cycle (90 d), survival (50%) and average weight at harvest (22 g) with respect to all 70 ponds studied, were used to produce dichotomous variables. Values equal to or less than the median were considered as crop failures. Yates' corrected chi-square test was used to test univariate associations with outcome variables. A p-value of 0.05 or lower was considered to be significant. All analyses were conducted using Epi Info 6.04b (Centers for Disease Control and Prevention, USA/World Health Organisation, Geneva, Switzerland) and Win Episcope 2.0 (N. de Blas, C. Ortega, K. Frankena, J. Nordhuizen & M. Thrushfield pers. comm.).

RESULTS

Details of samples collected and farmer compliance

A total of 56 of the 70 ponds recorded mortalities; 73 samples for histology were provided from 50 ponds and 55 samples from 44 ponds for PCR. Table 1 contains details of the samples.

Histopathology

Thirty-six samples of dead shrimp from 32 ponds (64%) showed clear evidence of WSD. This was in the form of hypertrophied nuclei with basophilic intranuclear viral inclusion bodies in the cells of ectodermal and mesodermal tissues such as the cuticular epidermis (Fig. 1), connective tissue (Fig. 2), gills, antennal gland, and other organs. The severity of infection was moderate to heavy, suggesting that WSSV infection was the cause of death. Post-mortem changes, although present in some of the samples, did not obscure the characteristic WSD diagnostic pathology (Fig. 3). Intranuclear inclusion bodies of WSD were still intact in the sloughed cellular debris in some of the dead samples (Fig. 4).

Table 1. *Penaeus monodon*. Details of samples provided by farmers. Histological samples = 73 from 50 ponds, PCR samples = 55 from 44 ponds

PCR samples	Histology samples
1 from each of 36 ponds	1 from each of 36 ponds
2 from each of 6 ponds	2 from each of 9 ponds
3 from 1 pond	3 from each of 2 ponds
4 from 1 pond	4 from each of 2 ponds
	5 from 1 pond
1–20 pleopods per sample	52 samples of (1–4 shrimp)
	18 samples of (5–8 shrimp)
	3 samples of (10–13 shrimp)

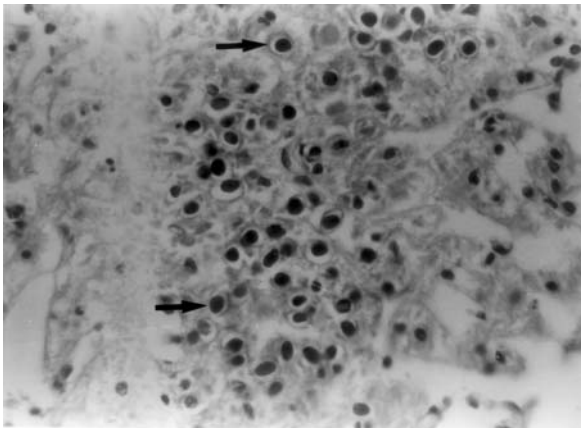


Fig. 2. *Penaeus monodon*. Intranuclear inclusion bodies (arrows) in the connective tissue of dead shrimp. H&E (×40)

Thirty-seven samples of dead shrimp from 18 ponds (36%) showed no histopathological evidence of WSSV infection. However, of these, 27 samples collected from 13 ponds (26%) showed clear evidence of shell, oral, enteric or systemic chronic inflammatory lesions (CIL) in the form of haemocytic aggregations and nodules. Such lesions are usually associated with chronic bacterial infections (Figs. 5 to 8). The extent of infection suggested that bacterial infection was the cause of death in these individuals. Post-mortem changes did not affect the diagnosis, as it was based on the evidence of inflammatory response and not on the presence of bacteria. A total of 5 samples from 5 ponds (10%) were negative for both WSSV and bacterial infection.

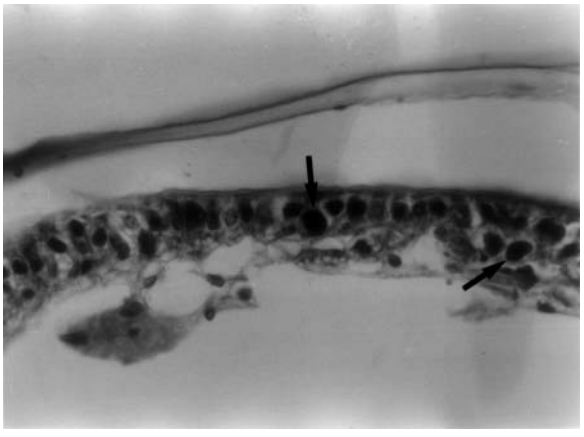


Fig. 1. *Penaeus monodon*. Intranuclear inclusion bodies (arrows) diagnostic of white spot syndrome virus (WSSV) infection in the cuticular epithelium of dead shrimp. H&E (×40)

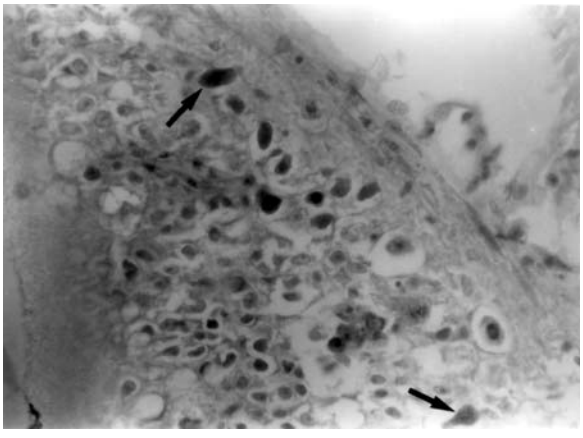


Fig. 3. *Penaeus monodon*. Irregular shaped intranuclear inclusion bodies (arrows) in the cuticular epithelium of dead shrimp. H&E (×40)

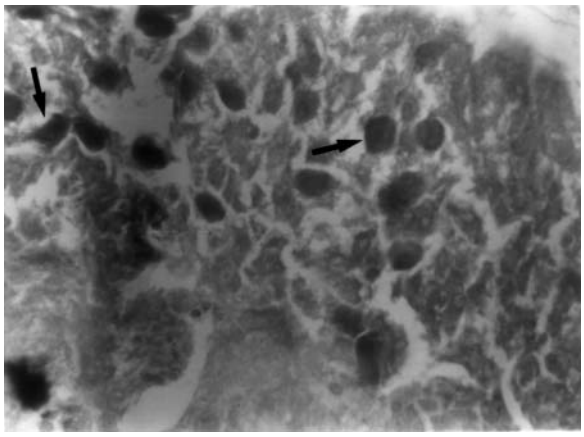


Fig. 4. *Penaeus monodon*. Intact intranuclear inclusion bodies (arrows) in the sloughed cells of foregut cuticular epithelium of dead shrimp. H&E (×40)

On a pond basis, histopathological evidence for mixed WSSV and CIL was observed in 8 samples from 8 ponds. However, WSSV and CIL occurred in the same shrimp in only 1 pond.

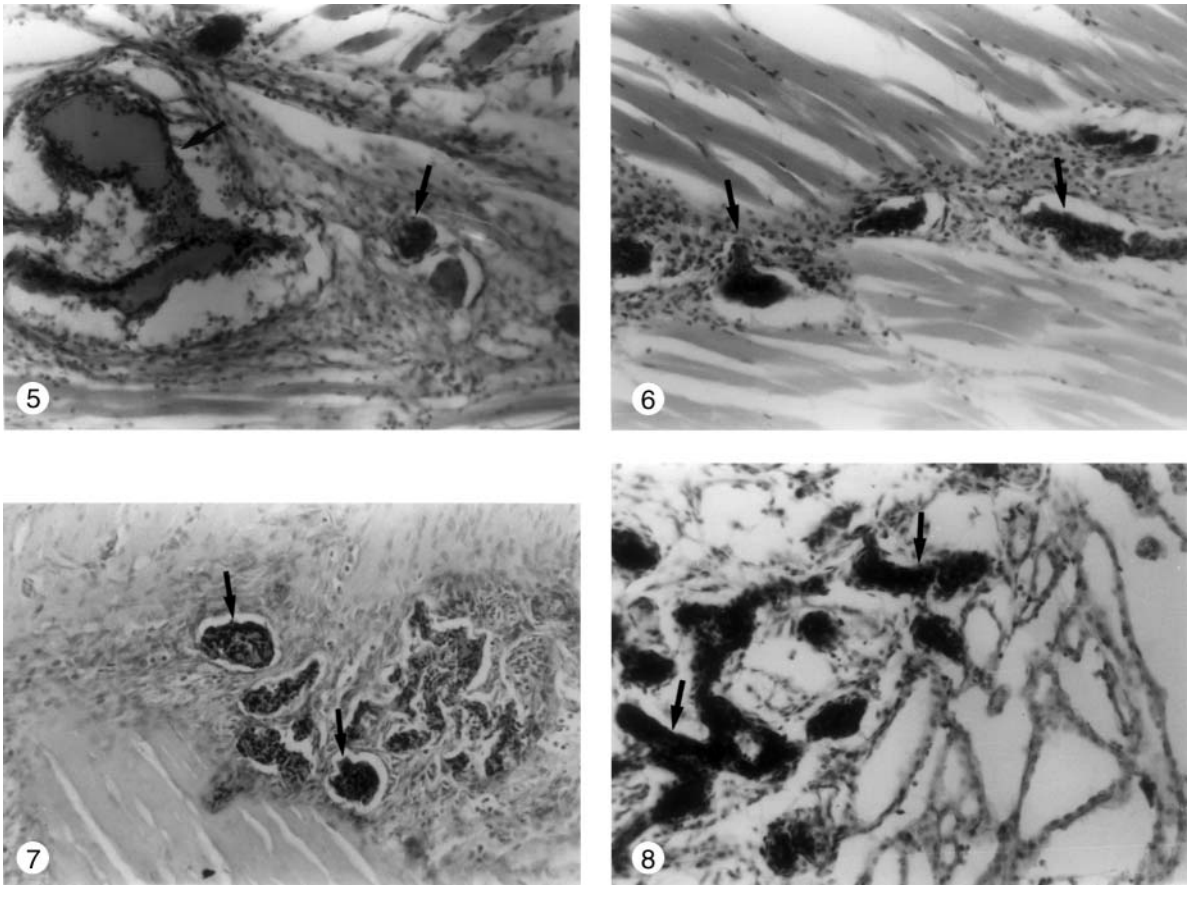
Fouling, ranging from light to very heavy was observed in all samples. As fouling is very common on moribund and dead animals, it was not considered in any of the analyses.

PCR

Twenty-one samples from 19 ponds (43%) were 1-step PCR positive, 12 from 10 ponds (22.7%) were positive only by 2-step nested PCR, and 22 from 15 ponds (34.1%) were negative by 2-step nested PCR for WSSV. The limit of detection assay showed that the 2-step nested PCR could detect WSSV DNA up to a dilution of $1:10^6$ for a 1-step positive pleopod from dead shrimp, whereas, it could detect up to a dilution of only $1:10^2$ for a 2-step positive pleopod (Thakur 2001).

Comparison between histopathology and PCR

The histopathology and PCR results were examined on a sample (55) and pond (44) basis to compare these



Figs. 5–8. *Penaeus monodon*. Chronic inflammatory lesions (CIL) in the form of: Fig. 5. haemocyte aggregations, nodule formation and melanisation (arrows) in the oral region; Fig. 6. cephalothoracic muscle; Fig. 7. abdominal muscle; and Fig. 8 antennal gland of dead shrimp. H&E (×20)

2 diagnostic methods for detecting WSSV in samples of dead shrimp. There was moderate to substantial agreement between PCR results and histopathology and this was statistically significant (Tables 2 & 3).

The proportion of ponds revealed as WSSV-positive by histopathology was similar, whether PCR samples were available or not (Table 4).

Association with outcome variables

Ponds with dead shrimp containing WSSV intranuclear inclusions were more likely to have crop failures as defined by a shorter production cycle, and lower average weight at harvest ($p < 0.001$). They were also more likely to have lower shrimp survival, although this was not significant ($p = 0.141$: Table 5). Ponds with dead shrimp that proved positive for WSSV by 2-step nested PCR were more likely to have a crop with a lower average shrimp weight at harvest ($p = 0.012$). These ponds were also more likely to have a reduced length of the production cycle, but this association was not significant ($p = 0.052$). There was no association between the 2-step nested PCR result and shrimp survival ($p = 0.828$: Table 6).

Ponds with dead shrimp that were positive for CIL were more likely to have successful crops, as defined

Table 2. *Penaeus monodon*. Comparison of agreement between 2-step nested PCR and histopathology results on the basis of samples and ponds

Basis	2-step PCR +ve	2-step PCR –ve	Kappa coefficient
Sample			
WSSV inclusions			
+ve	27	4	0.627
–ve	6	18	p < 0.001
Pond			
WSSV inclusions			
+ve	24	4	0.552
–ve	5	11	p < 0.001

Table 3. *Penaeus monodon*. Comparison of agreement between 1-step PCR and histopathology results on the basis of samples and ponds

Basis	1-step PCR +ve	1-step PCR –ve	Kappa coefficient
Sample			
WSSV inclusions			
+ve	20	11	0.576
–ve	1	23	p < 0.001
Pond			
WSSV Inclusions			
+ve	18	10	0.518
–ve	1	15	p < 0.001

Table 4. *Penaeus monodon*. Presence/absence of WSSV inclusions on a pond basis related to availability of PCR samples

PCR samples	WSSV inclusions +ve	WSSV inclusions –ve	
Provided	28	16	
Not provided	4	2	p = 0.631

Table 5. *Penaeus monodon*. Association of WSSV inclusions in dead samples on a pond basis, with length of production cycle, average weight at harvest and survival. OR: odds ratio; CI: 95% confidence interval

Parameter	Failure	Success	Statistic
Length of cycle	**<90 d**	**>90 d**	
WSSV inclusions			
+ve	24	8	OR = 10.50
–ve	4	14	CI = 2.27–53.47
			p < 0.001
Avg harvest weight	**<22 g**	**>22 g**	
WSSV inclusions			
+ve	24	7	OR = 11.44
–ve	4	13	CI = 2.31–59.58
			p < 0.001
Survival	**<50%**	**>50%**	
WSSV inclusions			
+ve	19	13	OR = 2.92
–ve	6	12	CI = 0.75–11.75
			p = 0.141

Table 6. *Penaeus monodon*. Association of 2-step nested PCR WSSV-positive infections in dead samples on a pond basis, with length of production cycle, average weight at harvest and survival. OR: odds ratio; CI: 95% confidence interval

Parameter	Failure	Success	Statistic
Length of cycle	**<90 d**	**>90 d**	
WSSV PCR			
+ve	20	9	OR = 4.44
–ve	5	10	CI = 0.99–21.18
			p = 0.052
Avg harvest wt	**<22 g**	**>22 g**	
WSSV PCR			
+ve	21	6	OR = 7.00
–ve	5	10	CI = 1.42–37.55
			p = 0.012
Survival	**<50%**	**>50%**	
WSSV PCR			
+ve	15	14	OR = 0.94
–ve	8	7	CI = 0.22–3.90
			p = 0.828

Table 7. *Penaeus monodon*. Association of chronic inflammatory lesions (CIL) in dead samples on a pond basis with length of production cycle, average weight at harvest and survival. OR: odds ratio; CI: 95% confidence interval

Parameter	Failure	Success	Statistic
Length of cycle	**<90 d**	**>90 d**	
CIL +ve	5	16	OR = 0.07
CIL −ve	24	5	CI = 0.01–0.31
			p < 0.001
Avg harvest wt	**<22 g**	**>22 g**	
CIL +ve	6	15	OR = 0.09
CIL −ve	22	5	CI = 0.02–0.42
			p < 0.001
Survival	**<50%**	**>50%**	
CIL +ve	8	13	OR = 0.43
CIL −ve	17	12	CI = 0.12–1.50
			p = 0.252

by a longer production cycle and higher average weight at harvest ($p < 0.001$). They were also more likely to have higher shrimp survival, but the association was not significant ($p = 0.252$: Table 7).

DISCUSSION

Farmer compliance

As this was a part of a larger study, the regular presence of the research assistants in the study area probably contributed to the high level of farmer compliance. This high compliance should have provided a reasonably precise estimate of prevalence of WSSV and CIL in the population. The approach adopted here for collecting dead shrimp during the production cycle appears to be practical, and was inexpensive.

Histopathological findings

Samples of dead shrimp from 32 ponds showed clear histopathological evidence of WSSV infection. Post-mortem changes when present did not affect the diagnostic features of WSSV. However, the irregular shape of intranuclear inclusions and the occurrence of multiple inclusion-like bodies could have been the result of post-mortem changes. Samples from 18 ponds did not show any evidence of WSSV infection, but samples from 13 of these ponds showed evidence of CIL. It is probable that CILs are indicative of chronic bacterial infections and this may provide a useful tool for diagnosis of such infections. Using the inflammatory response in the form of haemocytic nodules as an indirect diagnostic feature for bacterial infection is a good way of overcoming the problems associated with post-mortem changes. In 5 ponds, there was no evidence of any infection and it was not possible to ascertain the cause of death in these shrimp. For 4 of these ponds there was a very small sample size. On a pond basis, 8 ponds had evidence of dual WSSV and CIL, but in 7 of these the dual infection was not observed in the same shrimp. The observation that WSSV and CIL rarely occurred together in the same shrimp requires further investigation to explore the possible interactions between the 2 conditions.

Samples of dead shrimp from the edges of a pond may not be suitable for diagnosing the cause of mortality. However, the information from this project does suggest that dead shrimp can be used for diagnosis of WSSV inclusions and possibly chronic bacterial infections, since they produce distinctive lesions that are not easily confused with or masked by post-mortem change. Examination of dead samples allows one to distinguish the presence or absence of these lesions and make some degree of severity grading. The histological analysis described here would not be possible for other viruses like yellow-head virus (YHV), monodon baculovirus (MBV), baculovirus midgut gland necrosis (BMN) and hepatopancreatic parvovirus (HPV), in which degenerative post-mortem changes are more likely to mask the diagnostic pathology.

PCR findings

The PCR results indicated that samples of dead shrimp from 19 ponds were positive for WSSV by 1-step PCR, and an additional 10 ponds were positive when also tested by 2-step nested PCR. Samples collected from 15 ponds showed no evidence of WSSV infection. As PCR is highly specific (unlike histopathology), the cause of death in samples from the 15 negative ponds could not be determined. This is a limitation in using PCR alone in disease surveillance studies. The results suggest that testing dead shrimp can be effective in screening for WSSV. It is doubtful whether PCR or RT-PCR analysis on dead shrimp would work for RNA viruses because of the labile nature of RNA.

Level of agreement between PCR and histopathology

The 2 diagnostic methods used here agreed significantly in the diagnosis of WSSV infection. In some cases the samples with inclusions did not test positive by PCR (more so in 1-step analysis) and some PCR-positive samples did not have inclusions (more so

in 2-step analysis). There could be several reasons for such disagreement, including sampling errors such as failing to collect the legs from all the shrimp fixed for histopathology. According to Lo et al. (1996b), in most cases, positive PCR results coincide with WSD histopathology. Heavily infected shrimp usually test 1-step PCR-positive (Kou et al. 1998) and are likely to have detectable intranuclear inclusions in the cells of target tissue. However, histopathology may not always detect inclusions in a 2-step nested PCR-positive sample. This could be due to the low level of infection within particular shrimp and the absence of inclusions in such individuals. For PCR, only swimming legs were used as samples, and this is still the preferred target tissue. During the viraemic phase of infection, the virus may be present in many organs, and the level of viraemia may determine the level of infection in different target tissues (Kou et al. 1998). Variation in the distribution of viral inclusions might explain why some samples positive by histopathology were negative by PCR. The effect of post-mortem changes on viral DNA and its subsequent recovery for PCR is not known. A greater number of WSSV inclusion-positive samples in the present study were 1-step PCR negative than vice versa. This may suggest that the recovery of DNA from dead samples could have been lower, even below the detection limit of 1-step PCR. The better agreement between 2-step PCR and histopathology suggests that there was no significant influence of post-mortem effects on the nested PCR results.

Associations with outcomes

In this study, crop failures were associated with WSSV infection. It is possible that detection of dead shrimp in ponds and confirmation of WSSV infection could be used as predictors for crop failures. Although the 2 diagnostic methods provide similar prevalence estimates, histopathology appears to be a better predictor of WSD outbreaks than PCR.

Since there was an association between the presence of CIL and the absence of WSSV it is difficult to interpret the effect of CIL on the outcomes. Although there was an apparent protective effect of CIL, this may have been due to the absence of WSSV. CIL was very rarely observed with WSSV in the same shrimp. CIL (as observed in the present study) is typical of bacterial infections. The recent observation of Corsin et al. (2001) on the association between a reduced risk of WSSV in shrimp at harvest and the presence of high numbers of shrimp with external clinical signs of bacterial infection is interesting. These findings from population-based investigations underline the need for further studies to examine the possibility of CIL/chronic bacterial infection having a protective effect against WSSV.

Utility for epidemiological studies

The above findings clearly illustrate the usefulness of dead shrimp samples in epidemiological studies investigating WSSV and CIL. With proper farmer training, a high level of compliance can be achieved, and it should be possible to collect a large proportion of the dead shrimp. Such an approach would be cost-effective and practical for conducting large-scale disease surveillance and aquatic epidemiological studies in which time, money and manpower are major constraints.

Acknowledgements. We are grateful to Richard Callinan, Pornlerd Chanratchakool, Michael Phillips, Ian MacRae and Rohana Subasinghe who helped during the preliminary planning of the study. We would like to thank the College of Fisheries, University of Agricultural Sciences, Bangalore, for implementing the project. We would like to thank also the Brackish Water Fish Farmers Development Agency for supplying the farmers list. A special thanks goes to Iqlas Ahmed for his contribution in setting up the study and for helping during its progress. We would like to thank also all the farmers and hatcheries for extending their support to the study. This study was supported by the Department for International Development through project R7051 of the Strategy for Research on Renewable Natural Resources Program.

LITERATURE CITED

Anonymous (1997) The blight of Asian farms. Fish Farming Int 24:32

Calderon J, Bayot P, Betancourt I, Alday de Graindorge V (2000) Monitoring study of white spot syndrome in Ecuador. Abstracts, World Aquaculture Society, Nice, (Spec Publ No. 28)

Chou HY, Huang CY, Wang CH, Chiang HC, Lo CF (1995) Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Dis Aquat Org 23:165–173

Chou HY, Huang CY, Lo CF, Kou GH (1998) Studies on transmission of white spot syndrome associated baculovirus (WSBV) in *Penaeus monodon* and *P. japonicus* via waterborne contact and oral ingestion. Aquaculture 164: 263–276

Corsin F, Turnbull JF, Hao NV, Mohan CV, Phi TT, Phuoc LH, Tinh NTN, Morgan KL (2001) Risk factors associated with white spot syndrome virus infection in a Vietnamese rice-shrimp farming system. Dis Aquat Org 47:1–12

Flegel TW, Alday Sanz V (1998) The crisis in Asian shrimp culture: current status and future needs. J Appl Ichthyol 14:269–273

Kanchanaphum P, Wongteerasupaya C, Sitidilokratana N, Boonsaeng V, Panyim S, Tassanakakon A, Withyachumnarnkul B, Flegel T (1998) Experimental transmission of white spot syndrome virus (WSSV) from crabs to shrimp, *Penaeus monodon*. Dis Aquat Org 34:1–7

Kasornchandra J, Boonyaratpalin S, Itami T (1998) Detection of white spot syndrome in cultured penaeid shrimp in Asia: microscopic observation and polymerase chain reaction. Aquaculture 164:243–252

Kou GH, Peng SE, Chiu YL, Lo CF (1998) Tissue distribution of white spot syndrome virus (WSSV) in shrimp and crabs. In: Flegel TW (ed) Advances in shrimp biotechnology. National Centre for Genetic Engineering and Biotechnology, Bangkok, p 267–271

Lightner DV (ed) (1996) A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquaculture Society, Baton Rouge, LA

Limsuwan C (1997) Reducing the effects of white spot baculovirus using PCR screening and stressors. Newsl Aquat Anim Health Res Inst 6:1–2

Lo CF, Ho CH, Peng SE, Chen CH and 7 others (1996a) White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. Dis Aquat Org 27:215–225

Lo CF, Leu JH, Ho CH, Chen CH and 8 others (1996b) Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Dis Aquat Org 25:133–141

Maeda M, Itami T, Furumoto A, Hennig O and 5 others (1998) Detection of penaeid rod shaped DNA virus (PRDV) in wild caught shrimp and other crustaceans. Fish Pathol 33:373–380

Mohan CV, Shankar KM, Kulkarni S, Sudha PM (1998) Histopathology of cultured shrimp showing gross signs of yellow head syndrome and white spot syndrome during 1994 Indian epizootics. Dis Aquat Org 34:9–12

Momoyama K, Hiraoka M, Nakano H, Koube H, Inouye K, Oseko N (1994) Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus*, in Japan in 1993: histopathological study. Fish Pathol 29:141–148

Mushiake K, Shimizu K, Satoh J, Mori K, Arimoto M, Ohsumi S, Imaizumi K (1999) Control of penaeid acute viremia (PAV) in *Penaeus japonicus*: selection of eggs based on the PCR detection of the causative virus (PRDV) from receptaculum seminis of spawned broodstock. Fish Pathol 34: 203–207

Nakano H, Koube H, Umezawa S, Momoyama K, Hiraoka M, Inouye K, Oseko N (1994) Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus*, in Japan in 1993: epizootiological survey and infection trials. Fish Pathol 29: 135–139

Park JH, Lee YS, Lee S, Lee Y (1998) An infectious viral disease of penaeid shrimp newly found in Korea. Dis Aquat Org 34:71–75

Sudha PM, Mohan CV, Shankar KM, Hegde A (1998) Relationship between white spot syndrome virus infection and clinical manifestation in Indian cultured penaeid shrimp. Aquaculture 167:95–101

Thakur PC (2001) Estimation of white spot syndrome virus prevalence by polymerase chain reaction (PCR) in *Penaeus monodon* post larvae stocked into shrimp farms: emphasis on the methodology of detection. MFSc thesis, Department of Aquaculture, University of Agricultural Sciences, Bangalore

Thrushfield M (1986) Veterinary epidemiology, 2nd edn. Blackwell Science, London

Wongteerasupaya C, Vickers JE, Sriurairatana S, Nash GL and 6 others (1995) A non-occluded systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn *Penaeus monodon*. Dis Aquat Org 21:69–77

Zhan WB, Wang YH, Fryer JL, Yu KK, Fukuda H, Meng QX (1998) White spot syndrome virus infection of cultured shrimp in China. J Aquat Anim Health 10:405–410

Editorial responsibility: Chris Baldock, Brisbane, Australia

Submitted: July 8, 2001; Accepted: January 16, 2002
Proofs received from author(s): May 28, 2002