中山大学学报（自然科学版）
第 39 卷 增 刊 ACTA SCIENTIARUM NATURALIUM Vol. 39 Suppl.
2000 年 3 月 UNIVERSITATIS SUNYATSENI Mar. 2000

Article ID: 0529-6579（2000）S-0147-07

Theory and Strategies for Controlling White Spot Syndrome（WSS）of Cultured *Penaeus monodon* in South China*

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CLC number: S945.5 **Document code:** A

White spot syndrome virus（WSSV）is the causative agent of white spot syndrome（WSS）of cultured penaeid shrimps. WSS breaks out and prevails in cultured penaeid shrimps in many countries and regions of the world, especially in southeast Asia. WSSV is the virus most severe damaging to the cultured penaeid shrimps in the world. At the present time, the control of the outbreaks of WSSV will recover and develop the penaeid shrimp cultures in China and even in the world.

In 1991, WSS was found in cultured *Penaeus monodon* in Shantou, Guangdong Province, China. And in 1992, the WSS was broke out in cultured penaeid shrimps in Shantou and Fujian Province. However, it rapidly extended to all coastal areas in China in 1993, caused enormous economic losses. Until 1999 the penaeid shrimp cultures in China was still unrecovered.

WSS spreaded to the east of Guangdong Province in 1992 and to the west in 1993. Since 1995, due to adoption of new culture patterns and techniques, the yield of cultured penaeid shrimps in Guangdong has increased.

1 Diagnosis of WSSV

The virions of WSSV were purified from the gill and epidermis of *P. monodon*, which were in-

* 基金项目：国家自然科学基金资助项目（39670577）；国家教育部博士点基金资助项目；广东省自然科学基金资助项目
收稿日期：1999-09-15 何建国（1962～），男，教授，博士生导师.

fected by WSSV and were cultured in South China. Viral genomic DNA was extracted from purified virions. A partial WSSV genomic DNA library was constructed and a 12 576 bp nucleotide sequence was obtained. According to the nucleotide sequence, we designed nucleus primers for one-step and two-step PCR, nucleus probes for dot hybridization, and in situ hybridization to detect WSSV.

1.1 One-step PCR

WSSV can be detected by one-step PCR in the shrimps which were infected via oral exposure to WSSV contaminated tissues or fomites after 21 hrs, but not in the shrimps showing no white spot on cuticle and red body syndrome.

1.2 Two-step PCR

WSSV can be detected by two-step PCR in the shrimps which were infected via oral exposure to WSSV contaminated tissues or fomites after 3.5 hrs. It can be used to detect latent WSSV in parent shrimps, eggs, post larva in hatching period and shrimps during cultured period.

1.3 Dot hybridization

WSSV can be detected by dot hybridization in the shrimps which were infected via oral exposure to WSSV contaminated tissues or fomites after 21 hrs, but not in the shrimps showing no white spot on cuticle and red body syndrome.

1.4 *In situ* hybridization

WSSV can be detected by *in situ* hybridization in the shrimp which were infected via oral exposure to WSSV contaminated tissues or fomites after 21 hrs, but not in the shrimps showing no white spot and red body syndrome.

2 Infection and transmission of WSSV

2.1 Infection routes

2.1.1 Infection via digestive tract. WSSV can be first detected by mean of two-step PCR in the hemolymph, stomach, cephalothorax muscle, gill and appendages of *P. monodon* infected by oral exposure to WSSV contaminated tissues or fomites after 3.5 ~ 4.0 hrs. It revealed that WSSV could not proliferate a generation within 3.5 hrs in digestive tracts or other tissues and organs, then release to hemolymph. Apparently, Virions were transmitted via digestive tracts to hemolymph and sensitive target organs.

2.1.2 Infection via outer skin or shell. Exposed to WSSV by immersion, *P. monodon* with integrated outer skin or shell do not exhibit white spot syndrome, WSSV was under latency. However, when outer skin or shell is wounded and concentration of WSSV in the water environment is high, the shrimps will be infected and die. The concentration of WSSV in the water environment is low, the shrimp will not die, WSSV will be under latency. The virus in the environment comes from the feces of living hosts or/and the hosts die of WSSV infection.

2.2 Transmission

The transmission of WSSV can be divided into two types, one is horizontal transmission, and the other is vertical transmission. In our study, the latter can be defined as that WSSV is transmitted from parent shrimps to post larva in the hatchery ponds before the post larva are put into cultured ponds.

2.2.1 Horizontal transmission.

(1) There are all kinds of hosts of WSSV. In South China, there have known 31 species, which are all arthropods. Based on our detection, the natural hosts of WSSV include cultured shrimps, wild shrimps and crabs. Due to perennial culture, forage animals have proliferated in cultured ponds and

regions, causing the large increase in species and number of the hosts of WSSV, which comprise the main infection source of the horizontal transmission of WSSV. It is rather difficult to eliminate this infection source.

(2) In cultured penaeid shrimps regions, there are a great number of hosts of WSSV. WSSV carried by the hosts can survive in the winter and transmit to the shrimps next year. The rate of WSSV carried by the hosts is 80% during cultured shrimps period and 60% during non-cultured shrimps period. Therefore the shrimp infection rate of WSSV is much higher in both periods. At present time, since the whole cultured regions are threatened by WSSV, it is very difficult to control the outbreaks of WSS through eliminating the infection source of horizontal transmission and cutting off the infection pathway.

(3) The number of WSSV hosts and the rate of WSSV infection are apparently different in shrimp cultured regions and non-cultured regions. In cultured season, the host infection rate of WSSV is more than 80% in the cultured regions, and less than 30% in the non-cultured regions. Therefore, we suggest that it is better not to form a much larger cultured region, which will result in the increase in species and number of hosts, thus avoiding the horizontal transmission of WSSV.

(4) During the outbreaks of WSS, WSSV was transmitted among the natural hosts according to the food chain. It is a common phenomenon in nature that crustaceans kill and eat one another between the same and the different species. When the penaeid shrimps body length reaches 3 ~ 4 cm, cultured shrimps can eat dead crabs. Crabs can also eat dead shrimps. After the stage of post larva, the shrimps can kill and eat one another. Once some cultured shrimps die of WSSV, the death rate of the shrimps in the whole pond is near 100% within 7 ~ 10 days. Horizontal transmission is the main pathway in cultured penaeid shrimps during the outbreaks of WSSV.

(5) Transmission through water. Transmission of virions via water is also an important pathway to cause the latent infection of WSSV. In water environment, the main source of infective WSSV is the feces of latent infected and sick hosts, the release of died hosts infected by WSSV. WSSV can infect shrimps through two ways. One is that WSSV attach to the artificial feeds, and infect shrimps via feeding. And the other way is that WSSV infects shrimps via outer skin or shell when shrimps are wound. Shrimps will die when the concentration of the virions in the water is high, or will be latency when the concentration of the virions in the water is low.

2.2.2 Vertical transmission

(1) WSSV carried by parent shrimps. Eighty-nine *P. monodon* and *P. vannamei* parent shrimps (female) imported from other country (12 in 1997 and 77 in 1999) were detected by the means of one-step and two-step PCR. WSSV was detected in 56 shrimps and the detection rate was 67%. It revealed that *P. monodon* and *P. vannamei* parent shrimps are usually infected by WSSV.

(2) WSSV carried by post larva. In 1997, 1998 and 1999, post larva of *P. monodon* (body length 1.0 ~ 1.2 cm) in 57 hatchery were obtained. The WSSV detection rate of the post larva was 0 via one-step PCR and 56% via two-step PCR. The WSSV detection rate of *P. vannamei* post larva in 5 hatchery was 60% via two-step PCR. It revealed that WSSV carried by parent shrimps is the important reason of the infection of post larva and the latent infection of cultured penaeid shrimps.

(3) WSSV mainly infect the connective tissue of the ovary of *P. monodon* and can be observed in the oocytes.

(4) Artificial infection of parent shrimps.

Twentey-two *P. monodon* female parent shrimps were artificial infected via injection in 1998 and 1999. Two female parent shrimps of 1998 layed eggs, but the eggs did not hatched, and was detected

to be infected by WSSV via two-step PCR. Sevel female parent shrimps of 1999 layed eggs, and the eggs were hatched to young shrimps. WSSV was detected in eggs and post larva via two-step PCR. The post larva were cultured in concrete ponds and died after 40 days. The shrimps of the control (non-artificial infected) were cultured and successfully came into the market.

The ovary was detected by electron microscope, PCR and nucleic acid probe hybridization. WSSV was detected in the mature ova. It provided the evidence that WSSV may be vertical transmitted via eggs.

The research results indicated that vertical transmission is important to WSSV, causing the latent infection of cultured shrimps, which brings difficulty to cultured penaeid shrimps.

3 Latent infection of WSSV

Latent infection is the basis and one of the precondition of the outbreaks of WSSV at present time.

3.1 A lot of hosts carried by WSSV with the latent infection

At present time, all species of hosts discovered show latent infection, and different hosts have different infection rate and sensitivity. The latent infection changing into acute infection has close relation to climate, nutrition and physical and chemical factors in the water environment.

3.2 The pathogenicity of WSSV to shrimps depends on dosage of virions, which is the basis of latent infection. When a appendage of a shrimp (body length 8 cm) showing WSS and moribund was eaten by a healthy shrimp (body length 8 cm), the latter died. When 1/100 appendage of a shrimp with WSSV was eaten by a healthy shrimp (body length 8 cm), 40% infected shrimps were died and 60% shrimps with WSSV showed latent. The latent infected shrimps with WSSV eating enough dosage of WSSV contaminated tissue caused 100% shrimps died. When shrimps are fed on artificial feeds which have been attached to WSSV via immersion, and when WSSV infect wound shrimps, the pathogenicity of WSSV to shrimps also depends on dosage of WSSV.

3.3 Taches and factors causing WSSV latent infection

(1) Vertical transmission is one of the important sources of the latent infection of WSSV to penaeid shrimps.

(2) In horizontal transmission, infection via outer skin or shell is another important source of the latent infection of WSSV to penaeid shrimps.

(3) The artificial feeds contaminated with WSSV or the hosts carrying WSSV, were eaten by shrimps, WSSV may be latency in the shrimps.

(4) The virulence of WSSV was slightly weaken and the resistance of shrimps was increase.

(5) The good environment and nutrition are the foundation of the latent infection of WSSV.

4 Factors related with the outbreaks of WSS

There are two ways of the outbreaks of WSSV. One way of the outbreaks of WSSV is that shrimps eat the hosts carrying WSSV enough to cause acute infection, shrimps eat the feeds attached by high concentration WSSV and wounded shrimps are infected WSSV via outer skin or shell. Another way of the outbreaks of WSSV is that latent infection in cultured penaeid shrimps changes into acute infection due to all kinds of factors.

4.1 Cold wave

The analysis of the relation between the cold waves in Guangdong Province in recent three years

and the outbreaks of WSS show that, the effect of cold air in April and May will induce the outbreaks of WSS. Cold wave will not induce WSS to break out in the shrimps cultured less than 20 days. However, it will effect the shrimps cultured more than 20 days severely. And the longer the culture period and body length, the higher rate of the outbreaks of WSS induced by cold wave.

4.2 Heavy rain

Heavy rain directly relates to the outbreaks of WSS when without typhoon. Heavy rain will not induce WSS to break out in the shrimps cultured less than 20 days. However, it will effect the shrimps cultured more than 20 days severely. And the longer the culture time, the higher rate of the outbreaks of WSS induced by heavy rain.

4.3 Typhoon

There are several times of typhoon in the coastal areas of Guangdong. And usually typhoon comes with downfall. Typhoon will not induce WSS to break out in the shrimps cultured less than 20 days. However, it will effect the shrimps cultured more than 20 days severely. And the longer the culture time, the higher rate of the outbreaks of WSS induced by typhoon.

4.4 Depth of water in the ponds

There is a close relation between the depth of water in the ponds and the outbreaks of WSS. The depth of water in the ponds is within 50～150 cm, The deeper the water, the later the outbreaks of WSS, and the longer the shrimp culture period.

4.5 The amount of changing water

Changing water in a great amount in the ponds will easily induce the outbreaks of WSS. In our investigation, WSS outbroke when the ponds were exchanged water once or several times exceeding the amount of 40% 10 days before.

4.6 Relation between latent infection and physical and chemical factors in water environment

The relation between physical- and chemical- factors in water and climate reveals that, in Guangdong, the higher the temperature, the higher the water temperature, the salinity and pH, and the lower the content of ammonia and nitrite. The water temperature is higher when blowing southern and eastern wind, and is lower when blowing boreas. When blowing southwestern, content of ammonia and nitrite in water of cultured ponds is the highest, and when blowing eastern wind ammonia and nitrite is the lowest. When the wind power more than 7～8 degree, and when in cloudy and rainy day, the content of ammonia and nitrite and sulfide is obviously higher.

4.7 Relation between physical- and chemical- factors in cultured water and infection degree of *P. monodon*-type baculovirus (MBV)

There is less investigation of the relation between physical and chemical factors in water environment and the infection degree of WSSV in shrimp body (the number of viruses in unit volume of individual). Our study shows that, the effect of ammonia nitrite and salinity to the infection degree of MBV is most distingish. The higher the contents of ammonia, nitrite and salinity, the higher the infection degree of MBV in *P. monodon*. It is also verified by the means of multi-factor analysis and regress analysis step by step that, the effect of ammonia and salinity to the infection degree of MBV is most distinguish. The next is nitrite. The cooperation of pH and ammonia can distinguish improves the infection degree of MBV. Climate can effect the infection degree of MBV via effecting the physical and chemical factors in water environment.

The relation formula of the infection degree of MBV in *P. monodon* andphysical and chemical factors in water environment is:

$$Y = -12.089X_1 + 1.216X_2 + 2.734X_3 + 0.528X_4$$

Y = infection degree of MBV, X_1 = nutrition, X_2 = salinity (‰), X_3 = ammonia (μg/L), X_4 = nitrite (μg/L).

It has been affirmed that during the culture process of shrimps, WSSV latent infection and WSS outbreak concerning physical and chemical factors in water environment is similar to that of MBV. The formula about MBV may be used as reference to control WSS. Because a large of hosts and existence of latent infection of WSSV, at present time, we need to increase host resistance via nutrition to the virus, and control the water environment to control WSS.

5 Control methods of WSSV

(1) Eliminate the infection source of vertical transmission.
(2) Cut off the infection pathways of vertical transmission.
(3) Eliminate the infection source and pathways of horizontal transmission.
(4) Strengthen the control of water quality.
(5) Adopt the culture pattern of low salinity.
(6) Adopt the culture manner of increasing oxygen.
(7) Adopt the culture pattern of adding fresh water.
(8) Use deep ponds to culture shrimps.
(9) Changing culture system.
(10) Carry out uniform management during the large—scale culture process.
(11) Use disinfector suitably to treat water.

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