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White Spot Syndrome Virus（WSSV）Infection to Crabs*

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Abstract: White spot syndrome virus（WSSV）was found across different shrimp species and in different Asian countries. The present study attempted to investigate the WSSV infection in crabs collected from diseased shrimp pond and experimental infected to swimming crab, *Portunus trituberculatus*. Using electron microscopy, fluorescent antibody technique and polymerase chain reaction all of them obtained the result of WSSV positive, i. e. the crabs collected from diseased shrimp pond, infection by injection and oral infection but not immersion infection were virus positive. This was indicated that crabs probably the virus carrier.

Keywords: white spot syndrome virus（WSSV）; crab

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During the epidemic periods of the WSSV disease, we often noted that in the ponds of the disease occurred, the crabs, mainly *Helice* spp., *Macrophthalmus* spp., *Hemigrapsus* spp., were also showing signs of disease, for they did not escape when we came close to them and dead crabs were frequently observed. At the time, we did not know that the crabs infected by the virus or not, nor the shrimp or crabs who diseased first. To answer the questions, it is basic to know that crabs are sensitive to the virus or not. In this paper, we infected the swimming crab（*Portunus trituberculatus*）experimentally with WSSV and examined the virus using electron microscopy, fluorescent antibody technique and polymerase chain reaction methods, and the crabs collected from shrimp pond were also detected.

1 Materials and Methods

1.1 Crab

Swimming crab, 2.4 cm×4.1 cm in average size was used for experimental infection.

1.2 Infection trials

Moribund *Penaeus chinensis* with white spot served as inoculum for the infection trial. Gills, lymphoid organ and stomach tissue of moribund shrimp about 2 g total were homogenized in 20 mL of 0.85% NaCl solution and then centrifuged at 2 000 g for 20 min at 4 ℃. The supernatant was then passed through 450 nm membrane filter[1]. The filtrates containing virus were injected intramuscularly

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into healthy swimming crab with each receiving 0.1 mL. Oral infection was feeding the animal with diseased shrimp tissues, three times a day. Immersion infection was dipping the animal into homogenate of diseased shrimp tissues, dilution at 10^3 (w/V), for 30 min. The control groups were carried out respectively. Throughout the experiment the water temperature was 24～26 ℃, salinity was 30×10^{-12} and the pH was 8.5.

1.3 Transmission electron microscopy (TEM)

Tissues from the crabs (experimental infected and diseased from shrimp pond) were prefixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer solution (PBS pH 7.4) for 2 h at 4 ℃, rinsed with cold PBS three times, then postfixed in 1% osmium tetroxide for 2 h, dehydrated in alcohol and embedded in Spurr's resin. The ultrathin sections were stained with uranyl acetate and lead citrate[2], then examined with electron microscope.

1.4 Fluorescent antibody technique (FAT)

Cryosections were prepared from tissues of crabs (experimental infected and diseased from shrimp pond) and the healthy as control. The cryosections were fixed with acetone for 20 min, anti-WSSV monoclonal antibody[3] as primary antibody were applied, the samples were incubated for 60 min at 37 ℃, then rinsed three times with phosphate buffered saline for 5 min. Secondary antibody, fluorescein isothiocyanate-conjugated sheep anti-mouse Ig serum (diluted 1∶200) was applied, following treatment as described. After washing, the slides were mounted in glycerol and examined by fluorescence microscope.

1.5 Polymerase chain reaction (PCR)

A pair of primer according to Kimura et al[4] was used for PCR. The template DNA was extracted from the detected crabs tissue. The amplification was performed with a GeneAmp PCR system 9700 (Perkin, Elmer). 30 μL of PCR mixture consisted of 10 mmol/L Tris-HCl, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTP, 1.5 units of Taq DNA polymerase, 2 μmol/L primers and the template DNA. The PCR reaction was performed: First cycle of 93 ℃ for 3 min, 57 ℃ for 1.5 min, 72 ℃ for 5 min, and then 29 cycles of 93 ℃ for 1 min, 57 ℃ for 1.5 min, 72 ℃ for 1 min, plus a final 5 min extension at 72 ℃ after the 30 cycles[5]. The PCR products were electrophoresed in 1.5% agarose gel and stained with ethidium bromide. Positive and negative controls were also carried out.

2 Results

By injection the experimental crab died in 9 days, high mortality happened at 6 days after injection (Fig. 1). By oral infection crab all died till 25 days, high mortality occurred at 15 days after

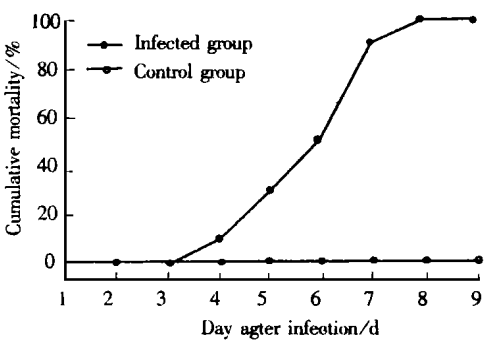

Fig.1 umulative mortality of crab infected by injection

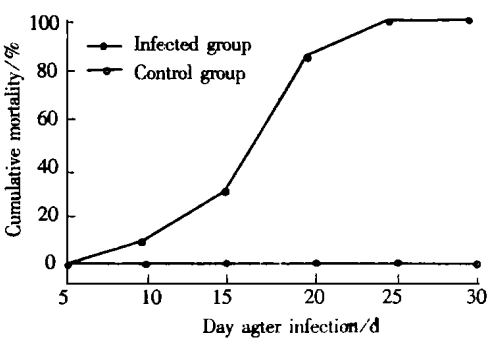

Fig.2 Cumulative mortality of crab infected by oral infection

infection (Fig. 2). None of crab was dead in immersion and control groups.

With TEM in the gill and epidermic tissues (Fig. 3) of infected crabs, viral virions were observed in nuclei of cells. The virions were same as found in diseased shrimp. By FAT in the tissues of infected crabs, the specific fluorescence was observed (Fig. 4 A), the fluorescence was not observed in tissues of immersion infection and control animals (Fig. 4 B). Using PCR the samples from injection and oral infection crab, the amplification DNA band appeared, no band appeared from the samples of immersion infection and healthy crabs (Fig. 5).

Fig. 3 Virions were observed in the nucleus of infected cell of crab. Scale bar = 600 nm.

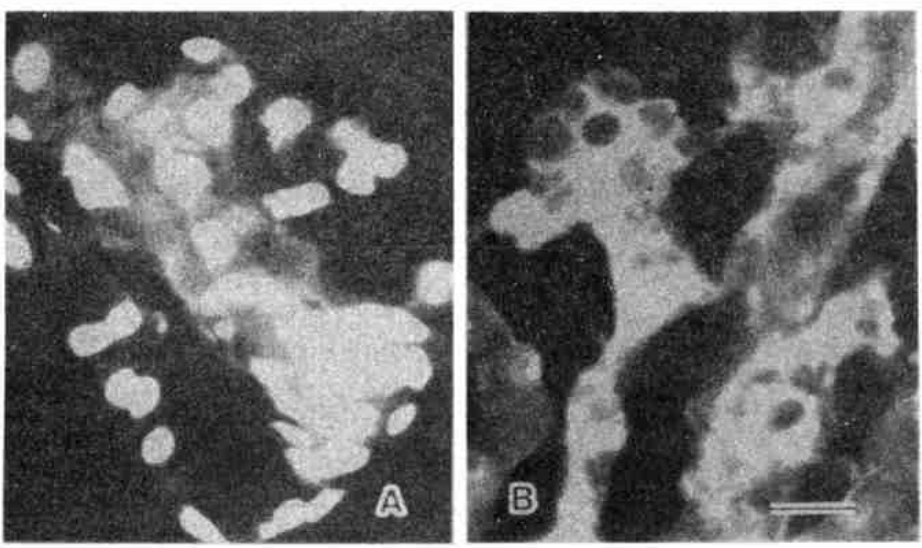

Fig. 4 Using FAT method

A infected crab tissue was virus positive; B healthy crab tissue was virus negative. Scale bar = 20 μm.

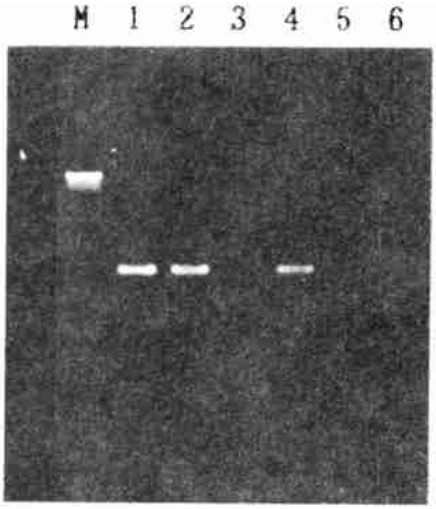

Fig.5 Detected the WSSV infection of crab by PCR method
1 injection infection,
2 oral infection,
3 immersion infection,
4 WSSV positive control,
5 healthy swimming crab,
6 WSSV negative control,
M marker.

3 Discussion

From the results of infection, swimming crab died in 9 days, high mortality happened at 6 days, while shrimp died in five days and high mortality happened at two to three days after injection[1]. By oral infection crab all died till 25 days, high mortality occurred at 15 days after infection, but shrimp did in a week. None of crab was dead in immersion infection. This indicated that swimming crab was less sensitivity to the virus than shrimp.

Although definite shrimp and crab viral infection relies on the examination of electron microscopy, but it is very time consuming. Diagnostic procedures for viral diseases of crustacean were largely dependent upon clinical signs, histological examination of moribund animals. The practical application of these procedures is sometime difficult and sensitivity is often limited[6]. Since 1993, biotechnological diagnoses using PCR, monoclonal antibodies, gene probes, such as those currently used in human and veterinary medicine, have been developed for some viral diseases of penaeid shrimp[3,4,6,7]. That made the rapid virus detection in shrimp become possible and those biotechnological diagnostic procedures are highly specific and have a high degree of sensitivity. In this paper we used three methods, TEM, FAT, PCR, all of them obtained the same result.

Using TEM, FAT, PCR methods, it was found that in the crabs (which were collected from the diseased shrimp pond and experimental infected) tissue the virus was same as WSSV. This was indicated that crabs probably virus carrier. Lo et al[7] found out that about 40% of pest crab *Helice tridens* and Palaemonidae prawn collected from epizootic areas of the virus infection were positive of the virus by 1-step diagnostic PCR.

Reference:

[1] ZHAN W B, YU K K, MENG Q X. Study on baculovirus disease of *Penaeus chinensis*[J]. J Fishery Science of China, 1995, 2: 22~28.

[2] ZHAN W B, WANG Y H, FRYER J L et al. White spot syndrome virus infection of cultured shrimp in China[J]. J Aquatic Animal Health, 1998, 10: 405~410.

[3] ZHAN W B, WANG Y H, FRYER J L et al. Production of monoclonal antibodies against white spot syndrome virus[J]. J Aquatic Animal Health, 1999, 11: 17~22.

[4] KIMURA T, YAMANO K, NAKANO H et al. Detection of penaeid rod-shaped DNA virus (PRDV) by PCR[J]. Fish Pathol, 1996, 31: 93~98.

[5] MOMOYAMA K, HIRAOKA M, INOUYE K et al. Mass mortality in the production of juvenile greasyback shrimp, *Metapenaeus ensis*, caused by penaeid acute viremia (PAV)[J]. Fish Pathol, 1997, 32: 51~58.
[6] DURAND S, LIGHTNER D V, NUNAN L M et al. Application of gen probes as diagnostic tools for white spot baculovirus (WSBV) of penaeid shrimp[J]. Dis Aquat Org, 1996, 27: 59~66.
[7] LO C F, HO C H, PENG S E et al. White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods[J]. Dis Aquat Org, 1996, 27: 215~225.

对虾白斑症病毒（WSSV）对蟹类的感染

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摘 要: 在对虾白斑症病毒（WSSV）病发生期间, 养殖虾池内的蟹类也往往出现异常甚至死亡. 通过 WSSV 对三疣梭子蟹（*Portunus trituberculatus*）的人工感染证实了其感染性. 三疣梭子蟹在注射感染后第 9 天全部死亡, 注射后第 6 天死亡率最高; 经口感染, 三疣梭子蟹在感染后第 25 天全部死亡, 在 15~20 d 死亡率最高; 在浸洗感染和对照组 30 d 内无蟹子死亡. 在感染死亡蟹子和发病虾池内病蟹的鳃、上皮组织的细胞核内电镜观察到的病毒粒子, 其形态特征与在对虾上观察到的一样. 用 PCR 和单克隆抗体的 FAT 法在注射感染和投喂感染的蟹子都得到了 WSSV 感染阳性结果, 浸浴感染的蟹子和健康蟹子都得到了 WSSV 感染阴性结果. 蟹类（三疣梭子蟹）对 WSSV 的敏感性较对虾低, 为此蟹类有可能是 WSSV 的携带者或传播者.

关键词: 白斑症病毒; 蟹类
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