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Viral Diseases of Some Species from Penaeid Shrimp in Iraqi marine waters

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Abstract

A total of 140 specimens of penaeid shrimps from *Metapenaeus affinis* and *Penaeus semisulcatus* were collected from different parts of Iraqi coastal waters to detect three species of viruses Infectious Hypodermal and Hematopoietic Necrosis virus (IHHNV), White Spot Syndrome Virus (WSSV) and Monodon Baculovirus (MBV). PCR and nest PCR used to identify the viruses. The main important results in this study were detection two of more important viruses, (IHHNV) and (WSSV). So, we thought the studied shrimp was not suitable for aquaculture stock without screening because which may play a vertical and horizontal vector role. This study represents the first attempt to detect viral shrimp diseases in Iraq.

Key Words: viruses, Shrimp, IHHNV, WSSV, MBV, PCR

Introduction:

Shrimp is one of the most important fisheries in the Arabian Gulf (Ramzy, 2001). It is one of the animals that play an important role in the economic and food supply of Iraq, especially in the Basrah province. The commercial fishing of shrimps in Iraqi coastal waters almost enclose with two main species, they are *M. affinis* and *P. semisulcatus* that have a high market value**.** Aquaculture accounted for 52% of world shrimp supplies in 2009. (Valderrama and Anderson, 2011). In 2010, the composition of the world aquaculture production was: freshwater fishes (65.4 %, 33.7 million t), mollusks (23.6 %, 14.2 million t), crustaceans (9.6 %, 5.7 million t), diadromus fishes (6.0 %, 3.6 million t), marine fishes (3.1 %, 1.8 million t) and other aquatic animals (1.4 %, 814 300 t). (FAO, 2012).

A lot of studies refer to the main problem in shrimp industry was disease outbreak, especially the viral diseases. The disease is one of most serious problem that faces this sector in the world and considered very important to

successful production in shrimp aquaculture (Johnson, 1995). Diseases represent the biggest obstacle to the future of shrimp farming and no medication to treat some disease like shrimp viruses and the prevention is the only remedy for viral infection (Weidner and Rosemberry, 1992). Disease outbreak has caused mass mortalities in many cultured penaeid shrimps worldwide especially in Asian countries (Kim *et al*., 1998). The disease results in partial or sometimes total losses of production, aquaculture in China suffered production losses of 1.7 million t caused by disease and outbreak of disease virtually wiped out marine shrimp farming production of Mozambique in 2011 (FAO, 2012). The culture of penaeid shrimp on wild-caught broodstock, which has an enormous potential to introduce new pathogens, particularly viruses into culture systems (Spann and Lester, 1997).

Materials and Methods:

A total of 140 specimens of shrimps including 80 of *M. affinis* and 60 of *P. semisulcatus* were collected from different parts of Iraqi coastal waters on a monthly basis from July to September, 2012 for the first species and from August to September, 2012 for the second species**.** Examined shrimp ranged from 4-14.1 cm length and 2-16 g weight for *M. affinis* and 11.2-17.3 cm and 18.6-44.3 gm for *P. semisulcatus*. Samples for PCR studies were collected and transported to the laboratory in chilled condition by using ice**.** For extracting DNA the Genomic DNA Mini Kit (Tissue) (Geneaid, USA) was used. The extracted DNA were checked by Electrophoresis and stored at -20^{o}C till further use. To amplification extracted DNA used the DNA Amplification Kit-DAK (Mangalore Biotech Laboratory, India). PCR amplification was carried out using the primers (table 1) for one step reaction to detect of IHHNV and MBV (one primer for each one), while two step reactions used to detect WSSV (nested PCR with two primers). positive control and negative control prepared for detection each IHHNV and MBV in one step reaction, and two step reaction for WSSV by PCR, that were performed using a thermal cycler . The contents were mixed well and subjected to thermocycling as in table (2).

PCR products were detected by using agarose gel electrophoresis. Depending on the size of the electrophoresis tank, the quantity of agarose was taken to prepare gel. 1.6 %(w/v) agarose gel were prepared in 1 X TAE buffer by heating. When the molten agarose was cooled to below 65 C^{o} and before pouring the gel, Ethidium bromide (five µl per 100 ml of gel) was added to final concentration of 0.5µg/ml and then mixed gently, after that, it was placed in the electrophoresis cast (Prakasha, 2003; Santiago *et al*., 2005). 10 µl of the amplification DNA were mixed with 4µl of 6X loading buffer and loaded into the wells. Molecular marker (100 bp DNA ladder, Mangalor Biotech. Laboratory, India) were used in a separate lane in each gel and run

with sample. The gel was run at 80 volts for 10 minutes and then at 120 volts. Then after electrophoresis, bands were visualized under UV transluminator (Herolab, Germany).

Table (1): The primers sequences that used and product size

Primer code	Primer sequence	Product size (bp)	Reference
MBV1.4NF/R	5'-TCCAATCGCGTCTGCGATACT-3' 5'-CGCTAATGGGGCACAAGTCTC- 3	361	Belcher and Young (1998)
IHHNV309F IHHNV309R	5'- TCCAACACTTAGTCAAAACCAA-3' 5'- TGTCTGCTACGATGATTATCCA - 3'	309	Tang *et. al.,* 2007
I step WSSV IK1 F IK2 R	 5'- TGGCATGACAACGGCAGGAG-3' 5'- GGCTTCTGAGATGAGGACGG -3'	486bp	Hossain *et al.*, 2001
II step WSSV IK3F IK4R	 5'-TGTCATCGCCAGCACGTGTGC -3' 5'-AGAGGTCGTCAGAGCCTAGTC -3'	310bp	

Table (2): Cycling conditions and expected product size

Primers	Cycling conditions					No. of cycles	Product size	Reference
	Initial delay	Denaturation	Annealing	Extention	Final delay			
I step IHHNV 309F/309R	94°C for 5min	94C° for 30sec	50C° for 30sec	72°C for 30sec	72°C for 5min	35	309bp	Tang *et. al.*, 2007
I step MBV 1.4NF/1.4NR	94°C for 5min	94C° for 30sec	60°C for 30sec	72°C for 30sec	72°C for 5min	30	361bp	Otta *et. al.*, 2003
I step WSSV IK1/2	94°C for 5min	94C° for 30sec	55°C for 30sec	72°C for 30sec	72°C for 5min	30	486bp	Hossain *et al.*, 2001
II step WSSV IK3/4	94°C for 5min	94°C for 30sec	55°C for 30sec	72°C for 30sec	72°C for 5min	30	310bp	

Results

The main important results in this study were the detection two of more important viruses, Infectious Hypodermal and Hematopoietic Necrosis virus (IHHNV) and White Spot Syndrome Virus (WSSV) as endemic viruses in Iraqi coastal waters. Another important result was the inability of using this species as stock for aquaculture directly without screening method because it was a carrier and infected with viruses that may be a vertical and horizontal transmission.

Infectious Hypodermal and Hematopoietic ecrosis (IHHN Disease)

A total of 47 samples (27) *M. affinis* and (20) *P. semisulcatus* were analyzed by PCR to detect IHHNV that causes this disease. One sample only gave a positive reaction for PCR from *M. affinis* specimens (Fig. 1), while all specimens of *P. semisulcatus* showed negative reaction for PCR. The total positive samples represent 3.7%; the infection was occurred (14.28%) in July, 2012 only (Table 3). The weight of infected specimen was less comparing with the other specimens and another clinical signs didn't noticed.

White Spot Syndrome Disease (WSSD)

A total of 47 samples, 27 of *M. affinis* and 20 of *P. semisulcatus* were analyzed by PCR to detect **(**WSSV)**.** All these samples showed a negative

reaction to one step PCR (Fig. 2) while the positive reaction for WSSV was in two steps PCR (nested PCR) (Fig. 3). 12 samples (14%) from the total samples gave a positive PCR reaction (Table 4). For *M. affinis,* the positive samples were 18.5% occurred in July, 2012 only (71.4%) and the positive samples of *P. semisulcatus* 35% occurred in August, 2012 only (77.77%). No clinical signs of disease were noticed in all the positive samples during the study period.

Monodon Baculovirus Disease (MBV disease)

A total of 46 sample (26) *M. affinis* and (20) *P. semisulcatus* were analyzed by PCR to detect MBV. All the samples showed a negative reaction to PCR.

Table (3): Detection of IHHNV for *M. affinis* and *P. semisulcatus* by PCR.

Month	Species	No. of Sample	No. of Positive	IHHNV	%
July	*M. affinis*	7	1	+	14.28
	P. semisulcatus	-	-	-	-
August	*M. affinis*	10	0	-	0
	P. semisulcatus	9	0	-	0
September	*M. affinis*	10	0	-	0
	P. semisulcatus	11	0	-	0
Total	*M. affinis*	27	1	+	3.7
	P. semisulcatus	20	0	-	0

Table (4): Detection of WSSV for *M. affinis* and *P. semisulcatus* by PCR and nest PCR.

Month	Species	No. of Sample	No. of Positive	WSSV First Step PCR	WSSV Second step (nested) PCR	%
July	*M. affinis*	7	5	-	+	71.4
	P. semisulcatus	-	-	-		-
August	*M. affinis*	10	0	-	-	0
	P. semisulcatus	9	7	-	+	77.77
September	*M. affinis*	10	0	-	-	0
	P. semisulcatus	11	0	-	-	0
Total	*M. affinis*	27	5	-	+	18.5
	P. semisulcatus	20	7	-	+	35

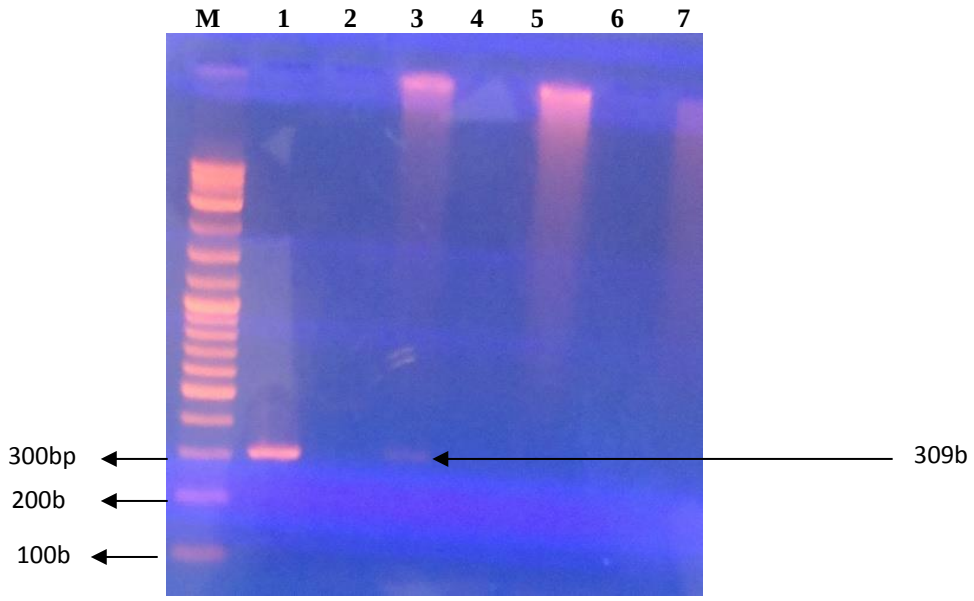

Fig. (1): Agarose Gel Showing PCR Amplification Product for IHHNV. Lane M: 100bp DNA marker; Lane 1: Positive control; Lane 2: Negative control; Lane 3: IHHNV positive sample PCR; Lane 4, 5, 6, 7: IHHNV negative samples for PCR.

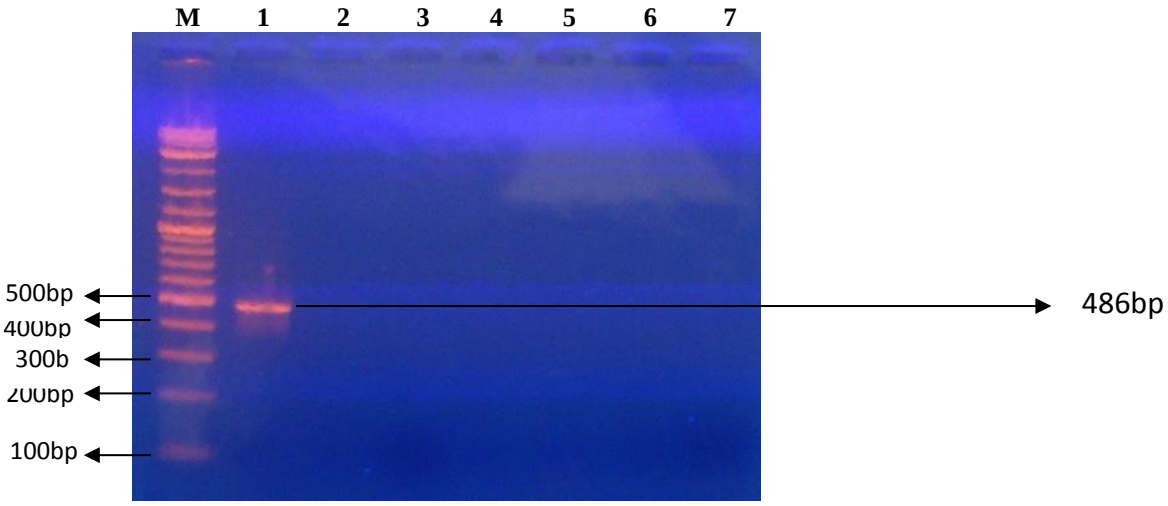

Fig. (2): Agarose Gel Showing PCR Amplification Product for WSSV (first step).
Lane M: 100bp DNA marker; Lane 1: Positive control; Lane 2: Negative control;
Lane 3-7: WSSV negative sample for I step PCR.

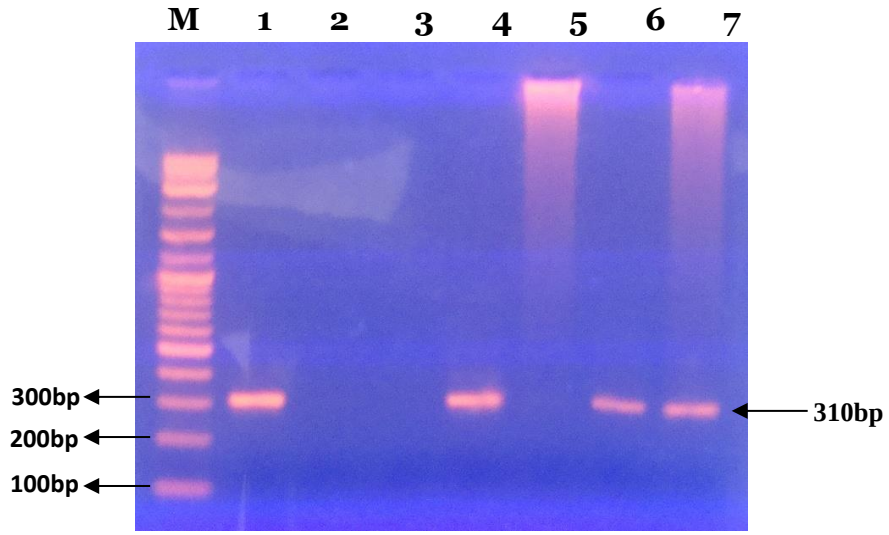

Fig. (3): Agarose Gel Showing PCR Amplification Product for WSSV (second step).
Lane M: 100bp DNA marker; **Lane 1**: Positive control; **Lane 2**: Negative control; **Lane 3, 5**: WSSV negative samples for nested PCR**; Lane 4, 6, 7**: WSSV positive samples for nested PCR.

Discussion

Since last three decades, shrimp farming has been the most profitable sector in aquaculture and the most important cultured species was from penaeid shrimp that belongs to the largest phylum Arthropoda in the animal kingdom (Sahoo *et al*., 2005). In this study, two species from penaeid shrimp that considered being more important in Iraq were collected to detect IHHNV, WSSV and MBV in Iraqi coastal waters. A virus is essentially a particle containing a core of nucleic acid, DNA or RNA, once inside a proper host cell, the viral nucleic acid interacts with that of a normal cell to cause reproduction of the virus, Certainly viruses cause important disease in particular circumstances but key understandings of most shrimps viruses are largely unknown (Johnson, 1995).

The results showed that the IHHNV and WSSV were endemic in Iraqi coastal waters through the positive reaction to PCR and nest PCR. Similar results were founded in different parts of the world. There were many reports about the record of these viruses in wild stock in America, South East Asia, North East Asia and Africa (Lightner, 1992; Prakasha, 2003). Our ability to detect shrimp viruses is ahead of our ability to evaluate their importance or to implement controls (Johnson, 1995). Information on the prevalence of shrimp pathogenic viruses in wild crustaceans would be important to understand the source of virus to culture system, in addition the viruses in wild crustaceans may impact infection of broodstock and the shrimp culture in Asia was depended on wild broodstock (Prakasha, 2003).

The effective control and treatment of diseases of aquatic animals required access to diagnostic tests that are rapid, reliable and highly sensitive and now DNA-based detection methods for detect of penaeid shrimp viruses are used routinely in a number of laboratories around the world (Walker and Subasinghe, 2000). PCR is an invitro method for specific DNA amplification and the purpose of PCR is to make huge number of copies of the desired gene (Snaker *et al*., 2011). Fegan (2000) attention to PCR test is a high sensitivity which reaches to 97% and this would be useful for screening to reduce the possibility of introducing infected individuals into a population.

Infectious Hypodermal and Hematopoietic Necrosis (IHHN disease)

Most penaeid species can be infected with IHHNV, including the principal cultured species but the sensitive species was Blue Shrimp *P. stylirostris* and caused mass mortality in juvenile and subadult life stage while this virus reduced growth rates in other species like *P. monodon* (OIE, 2006). Effect of this virus varies among penaeid species, for example shrimps such as *P. vanamei* and *P. monodon* were infected with IHHNV didn't show mortality (Karunasagar and Karunasagar, 2006). IHHNV was infected a wide range of

penaeid shrimps and natural infection have been reported in *P.vanamei*, *P. stylirostris, P. occidentalis, P. monodon, P. semisulcatus, P. califomiensis* and *P. japonicus* , but didn't appear to infect other decapods crustaceans (Lightner, 1996; Bondad-Reantaso *et al*., 2001).

The positive samples of *M. affinis* represented 3.7%. The infection occurred (14.28%) in July, 2012 only (Table 1). Agarose gel showed a light band of PCR amplification product for IHHNV. Low levels of infection and light band refer to the IHHNV was very low in the infected shrimp. Clinical signs were not noticed but the infected shrimp was small size (two cm only). Prakasha (2003) studied viral infection in wild for *Penaeus indicus*, *P. japonicas*, *P. semisulcatus*, *M. affinis*, *M. dobsoni*, *M. elegans*, *M. ensis*, *M. monoceros*, *Parapenaeus longipes* and *P.coromandelica* and didn't find any species as being infected with this virus.

White Spot Syndrome Disease (WSSD)

WSSV has become the most dangerous virus to the penaeid shrimp farming industry; this virus has virulent nature, wide host range, wide geographic distribution, high mortality and catastrophic economic losses (Karunasagar and Karunasagar, 2006). This virus was the causative agent of the white spot disease (WSD) and targets various tissues originating from mesoderm and ectoderm, particularly cuticular epidermis, gills, stomach, lymphoid organ, hematopoietic and antennal gland (Santiago *et al*., 2005).
High sensitive methods like PCR and nest PCR were used to detect the diseased shrimp and infected shrimp. In addition, using best primers (Ik1/2 and IK3/4) in this study increased the sensitivity. In the case of screening for carrier state infection, there was a need to use primers yielding smaller amplicons like Ik1/2 and IK3/4 (Hossain *et al*., 2004).

Both *P. semisulcatus* and *M. affinis* were infected. They showed a positive reaction for nest PCR only while all the samples a negative reaction for one step PCR. That means the positive samples for nest PCR were carrier (infected shrimp) for the virus; therefore, clinical signs were not noticed. Highly sensitive nested PCR was able to detect very low levels of infection and when PCR becomes positive by non-nested reaction that indication of progression of infection to the status of disease (Umesha, 2002). Many studies on viruses in wild caught shrimps had the similar result and mostly WSSV was showed positive reaction for nest PCR more than one step PCR (Otta *et al*., 1999; Prakasha, 2003; Hossain *et al*., 2001).The samples were positive for nest PCR only for two reasons. The first was a low level of infection with WSSV. The second was nest PCR which was more sensitive than PCR. Shariff *et al*. (2000) refers to the nest PCR was at least 100 to 1000 times more sensitive than single PCR.

The positive samples were high in both shrimp species. Positive samples occurred in July, 2012 only (71.4%) for *M. affinis* and in August, 2012 only (77.77%) for *P. semisulcatus*. All decapods (order Decapoda) crustaceans from marine and brackish or freshwater and all life stages were susceptible for this virus, in addition there were many vectors can accumulate high concentration of viable WSSV like bivalves, polychaete worms, non-decapodal crustacean including *Artemia salina* and the copepods, non-crustacean aquatic arthropodssuch as *Isopoda* and *Euphydradae* insect larvae (OIE, 2006). Generally, for these characters of this virus the result was expected. High level of infection in the July, 2012 for *M. affinis* and in August, 2012 for *P. semisulcatus* only may be belong to specimens collection site because of there were different site for caught shrimp off the Iraqi coast but surely this level refers to WSSV was endemic in Iraqi coastal environment. The probability of detection can be increased by exposure to stressful condition like spawning, changes in salinity and temperature (OIE, 2006). Prakasha (2003) studied the prevalence of infection of WSSV in wild caught shrimps in the west coast of India and was up to 50%, however the comparison with this study and others was not suitable because no activity for shrimp aquaculture in Iraq. Quang *et al.* (2009) referred to the relationship between outbreaks of this virus in shrimp ponds and surrounding areas. The high viral load could be due to the draining of the untreated infected effluents from the shrimp farm directly into the marine ecosystem (Sankar *et al.*, 2011), but couldn't depend on this relation because of the absence of shrimp farming in Iraq. Therefore, the prevalence of WSSV in July and August, 2012 was considered to be alarming rate.

Monodon Baculovirus Disease (MBV disease)

The International Committee on Virus Taxonomy lists the MBV as a tentative species in the genus *Nucleopolyherdovirus* and it was enzootic in wild penaeids in the Americas and Hawaii (OIE, 2006). Prakasha (2003) studied the prevalence of infection of MBV in wild populations of *M. monoceros*, *M. affinis, M. elegans* and *P. monodon* in west coast of India and was infected (4.13%), also was recorded in southwest coast of India in *M. elegans* and *M. monoceros* (Manivannan *et al.*, 2004).

MBV has not been found in any samples in this study but in the Middle East the MBV has been found in Kuwait and Oman (Lightner, 1996).

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100 المجلة العراقية للأستزراع المائي المجلد (12) العدد (2) – 2015: 88 – 100

الامراض الفايروسية لبعض انواع الروبيان البينايدي في المياه البحرية العراقية

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الخلاصة

تم جمع 140 عينة من روبيان البينايدي من النوعين *Penaeus semisulcatus* و *Metapenaeus affinis* من مناطق مختلفة من المياه البحرية العراقية لتشخيص ثلاث انواع من الفايروسات هي Infectious Hypodermal White Spot Syndrome Virus (WSSV) و Hematopoietic Necrosis virus (IHHNV) و Monodon Baculovirus (MBV) باستخدام تقنية تفاعل البلمرة التسلسلي PCR و nest PCR. النتائج الرئيسية التي تم الحصول عليها هو تشخيص نوعين من اهم الفايروسات هما (WSSV) و (IHHNV) لذا فان نوعي الروبيان لايمكن استخدامهما لاغراض الاستزراع بدون فحص وقائي للفايروسات اذ ربما تلعب دورا ناقلا للفايروسات بشكل طولي او عرضي. ان الدراسة الحالية تمثل المحاولة الاولى في العراق لتشخيص الامراض الفايروسية للروبيان.

كلمات مفتاحية: فيروسات، الروبيان، IHHNV, WSSV, MBV, PCR.