Prevalence and intensity of protozoan ectoparasite of the white leg shrimp (*penaeus indicus*) in Helleh site, South of Iran

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Abstract

Development of shrimp farming has been associated with the incidence of fatal diseases including viral, bacterial and protozoan parasites of shrimp. Our study was aimed to present a status of several important protozoan parasites of Litopenaeus van namei in cultured earthen ponds in Iran during the period of 2011-12. One hundred live shrimp were randomly prepared from the Shrimp Helleh Station in south of Iran. Prevalence and intensity were two important indices that calculated in our research. They were scraping, puttingon clean slides and examined under microscope to observe the protozoan parasites. *Zoothamnium* sp. is more prevalent pathogen among *Peritrichous ciliates* (79 to 88) in pleopods. *Acineta* sp. was less prevalent in our isolations neither in pleopods nor in gills. It was concluded that, *Peritrichous ciliates* are more frequent ciliates causing mortality in cultured shrimps at high density in higher temperature and ammonia.

Keywords: *Penaeus indicus* , protozoan, epibiont ciliates, epicommensal.

Introduction

In recent decades, Development of shrimp farming has been associated with the incidence of fatal diseases including viral, bacterial and protozoan parasites of shrimp (Lightner 1996). Therefore, shrimp farmers have been suffering from losing the production (Afsharnasab 2012; Kakoolaki, Sharifpour, Sharifrohani, Ebrahimzadeh Mousavi, Afsharnasab, Hoghoughirad, Dashtiannasab & Nezamabadi 2013). Shrimp production in Iran is not exempt from these global fluctuations. When shrimp production in 2007 fell by half, the farmers shifted the cultured species from Fenneropenaeus indicus to *Litopenaeus vannamei* to reach 20,000 tons in 2013 as highest record (Kakoolaki et al. 2013). The exoskeleton of crustaceans provides suitable organic substrates for many species of epibiont ciliates, especially suctorians, chonotrichs, and peritrichs (Mayen-Estrada R. & Aladro-Lubel M. A. 2002).

Some researchers have reported *Peritrichous ciliates* parasites such as *Zoothamnium* sp., *Epistylis* sp., *Acineta* sp. and *Vorticella* sp. in which exoskeleton are the target tissue (Jalali Jafari 1990; Kakoolaki 1997; Tamjidi 1995). Protozoan parasites and commensals occur both inside and outside the host body. *Zoothamnim* sp. and *Vorticella* sp. are ecto-parasites and gregarines is endo-parasite (Chakraborti & Bandyapadhyay 2011). Quantity of Peritrichs can rapidly increase, attach and feed on the exoskeleton and gill tissues due to poor quality of either earthen or hatchery pond water (Jalali Jafari 1990; Kakoolaki 1997). Overstreet (1973) showed that there is a putative relationship between the epibiont ciliate, *Zoothamnium* sp. and mortality of shrimp following stress. Their abundant presence can interfere with the breathing and mobility of the host (Jayasree, Janakiram & Madhavi 2001). *Zoothamnium* sp. are at their peak during climate change and increasing the concentration of nitrite or nitrate and can be controlled by salinity changes or habitat (Jayasree et al. 2001).

Zoothamnium is a frequent inhabitant of the gill surfaces of shrimp in ponds with low oxygen content and heavily infested shrimp can suffocate. Sur-

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face-settling protozoa occasionally cause problems in shrimp hatcheries when larval shrimp become overburdened and arc unable to swim normally. As protozoa continuously multiply in numbers, shrimp acquire anincreasing burden until shedding of the cuticle provides relief. Members of one unique group of protozoa, the apostome ciliates, have a resting stage that will settle on shrimp surfaces. When the crustacean molts, the protozoan releases and completes the life cycle within the shed cuticle before entering the stage on a new crustacean (Johnson 1990).

In this paper, we will attempt to present the status of several important protozoan parasites of shrimp in cultured earthen ponds in Iran.

Materials and Methods

Sampling

The present study was done during the period of 2011 to 2012. One hundred live shrimp Litopeneaus vannamei were randomly prepared from the Shrimp Helleh Station in south of Iran. They were transferred to National Shrimp Research Center in Bushehr provinceand then acclimated and reared under the optimum condition of water quality (Kakoolaki, Soltani, Ebrahimzadeh Mousavi, Sharifpour, Mirzargar, Afsharnasab & Motalebi 2011).

Isolation of pathogens

Animals were scraping from Exoskeleton and gills were taken on clean slides. The smeared slides were air dried, fixed in acetone free methanol and stained with Giemsa or directly wet smeared. The slides were examined under microscope to observe the protozoan parasites. From the collected data, prevalence and intensity of infection by each species of parasites were determined.

Prevalence and Intensity of infection

One hundred of shrimp were dedicated for collecting the ecto-parasites to identify the prevalence and intensity of infection.

Prevalence of infection was calculated using following formula:

P= N of infected shrimps/ all shrimps in an aquarium (Oie 2006)

Where Pis prevalence in an aquarium and N is number that given as Mean±SD.

Intensity of infection was analyzed as follows:(Malolahi A. & Mokhayer B. 2001)

I= If the number of observed parasites under light microscope were 5-50, 51-100 and more than 100, the intensity named mild, moderate and severe, respectively.

Statistical analysis

Cross tabulation, accompanying with Kendall's tau-bwas used to determine the relation between rows and columns with the probability of 95%.

Results

In cross-tabulation that was made between 2 subgroups of weight (under and above 15 g) and 6 groups containing 3 subgroups, only the relation between weight and *zoothamnium* in pleopods was significant (Kendall's tau-b: .003) and with other groups were not significant (p>0.05). Based on the table 1, it seems 66.7 % of infected pleopods were in the shrimp with the weightof less than 15 g.

In cross-tabulation is given in Table 2, there is a significant relationship (Kendall's tau-b: .026) between the occurrence of *Epistylis* sp. either in pleopods and gills of infected shrimp. But lesser or absent of severity in gills had significant relation with same protozoa in pleopods. According to the Table 2, it seems *Epistylis* were observed more in pleopods in comparison to gills. Based on the Table 3, there was a significant relationship (Kendall's tau-b: .048) between the occurrence of *Zoothamnium* sp. in pleopods and *Acineta* sp. in gills of infected shrimps. It showed that increasing the incidence of *Zoothamnium* sp. in pleopods has significant relation with lesser incidence of *Acineta* sp. of gills.

The prevalences of *Zoothamnium* sp., *Epistylis* sp. and *Acineta* sp. in pleopods were calculated as follows:

P (*Zoothamnium* sp.)= 79/ 100×100, P= 79 % (n: 100)

P (*Epistylis* sp.)= 61/ 100×100, P= 61 % (n: 100)

P (*Acineta* sp.)= 7/ 100×100, P= 2 % (n: 100)

The prevalences of *Zoothamnium* sp. ,*Epistylis* sp.

Table 1 Cross tabulation between weight groups and *Zoothamnium* sp. observed on pleopods and gills.

			Pleopod*Zoothamnium* sp.			Total
			Severe	moderate	mild	
Weight grouping	1	Count	9	8	7	24
		% of Total	25.0%	22.2%	19.4%	66.7%
	2	Count	9	3	0	12
		% of Total	25.0%	8.3%	.0%	33.3%
Total		Count	18	11	7	36
		% of Total	50.0%	30.6%	19.4%	100.0%

Table 2 Cross tabulation between *Epistylis* sp. in Pleopod groups and *Epistylis* sp. observed in Gills.

			Epistylis in Gills		Total
			3	4	
Epistylis in Pleopods	1	Count	9	5	14
		% of Total	25.0%	13.9%	38.9%
	2	Count	8	3	11
		% of Total	22.2%	8.3%	30.6%
	3	Count	2	9	11
		% of Total	5.6%	25.0%	30.6%
Total		Count	19	17	36
		% of Total	52.8%	47.2%	100.0%

Table 3 Cross tabulation between Zoothamnium sp. in Pleopodseverity groups and *Acineta* sp. severity groups observed in Gills.

			Acineta in gills		Total
			3	4	
Zoothamnium in Pleopods	Severe	Count	1	17	18
		% of Total	2.8%	47.2%	50.0%
	moderate	Count	2	9	11
		% of Total	5.6%	25.0%	30.6%
	weak	Count	3	4	7
		% of Total	8.3%	11.1%	19.4%
Total		Count	6	30	36
		% of Total	16.7%	83.3%	100.0%

and *Acineta* sp. in gills are given as follows:
P (*Zoothamnium* sp.)= 12/ 100×100, P= 12 % (n: 100)
P (*Epistylis* sp.)= 7/ 100×100, P= 7 % (n: 100)
P (*Acineta* sp.)= 5/ 100×100, P= 5 % (n: 100)
The figures of common and non-common *peritrichous ciliates* observed in shrimp are given as Tables 1-6. Whole figures show a common stalk for attaching to the host tissue.

Discussion

Our study was aimed to identify the ecto-parasite species and determined the prevalence and intensity of the infection in *Litopenaeus vannamei* cultured in Iran. According to the results obtained, the prevalence of *Zoothamnium* sp. was higher in comparison to *Acineta* sp. or *Epistylis* sp. In the other view, the *peritrichous ciliates* were more common in pleopods but gills. It seems shrimp with lesser weights were more susceptible to *Zoothamnium* sp. but no significant difference was observed between severities across the groups (Table 1).
According to the results of prevalences (Fig. 3), *Acineta* sp. was less prevalent in our isolations neither in pleopods nor in gills. Based on Table 2, there

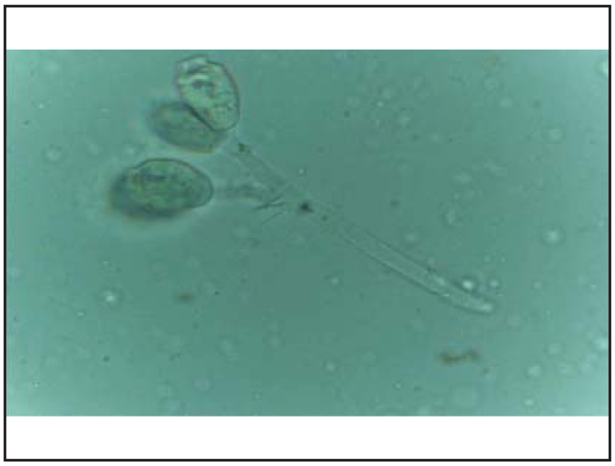

Figure 1 It shows 3 *Zoothamnium* with 3 individual stalks and 1 common stalk to attach to host tissue; wet mount, × 400.

Figure 2 It shows *Epistylis* with a common stalk to attach to host tissue; wet mount, × 400.

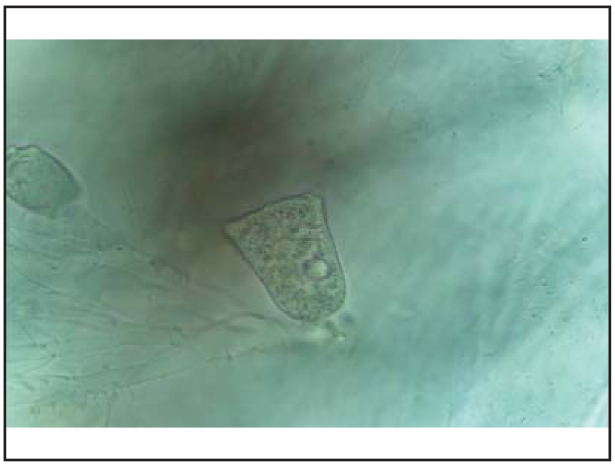

Figure 3 *Acineta* attached to a host tissue; wet mount, × 400.

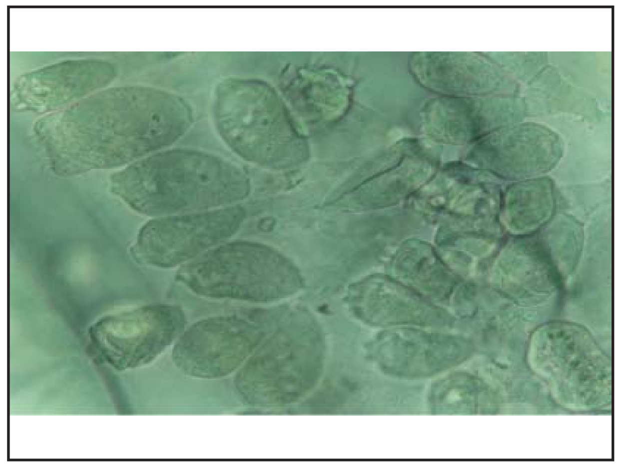

Figure 4 Intensity of attached different parasites to shrimp gill; wet mount, × 400.

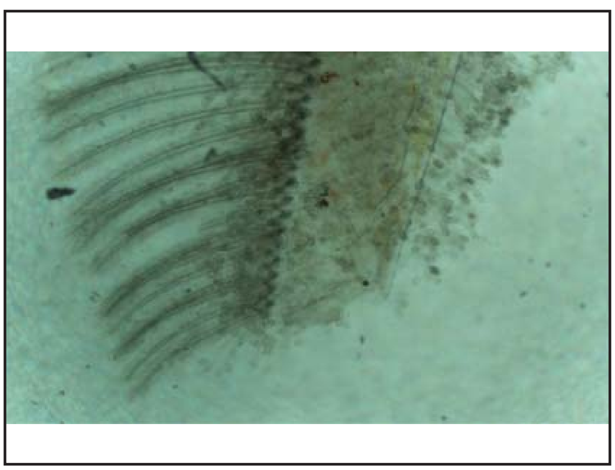

Figure 5 Intensity of attached different parasites to shrimp gill; wet mount, × 40.

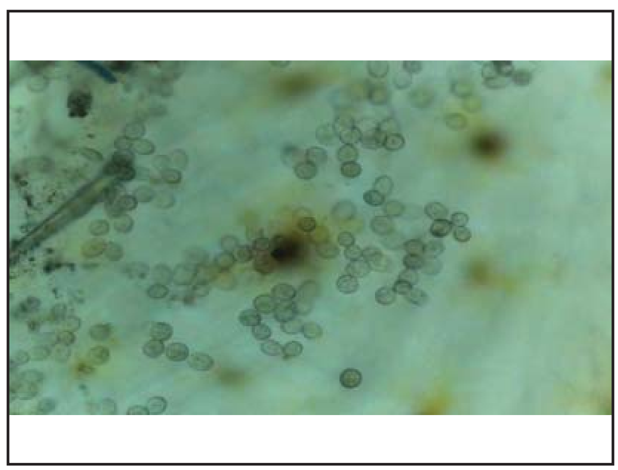

Figure 6 Intensity of attached different parasites to shrimp pleopods; wet mount, × 100.

is a matched trend between the severity of *Zoothamnium* sp. in pleopods and gills so that while increasing of the infection became more in pleopods, an increasing trend of the parasite incidence also was observed in gills. On the other hand, increasing of severity of *Zoothamnium* sp. in pleopodswas contrarily matched with *Acineta* sp. in gills. Similar to the result of Abedian & Ebrahimi (2006) our results

showed that *Zoothamnium* sp. is more prevalent pathogen among *peritrichous ciliates* (88.66% to 79%) in pleopods. The least value for shrimp ciliated infections belong to *Acineta* with 2% of shrimp pleopods. This result is contrary to the result of Abedian & Ebrahimi (2006) that recorded *Vorticella* sp. has least prevalence among ciliates (0.24%).
In another survey (Mayen-Estrada R. & Aladro-Lubel M. A. 2002) *Vorticella* sp. was the most prevalent peritrichous for crayfish attached to pereiopods and gills. This result is matched to the result of Chakraborti & Bandyapadhyay (2011) that confirmed *Vorticella* sp. the most frequent ciliates from tiger shrimp. They showed the infection increase at 29-31°C and decrease above or below this degree. Thus, an optimum temperature of 29–31°C is required for growth and survival of the protozoan parasites. Since they are epibionts, the infection caused by them at a low density is non pathogenic but heavy infection may cause fouling of gills and appendages. They may even cause death since they interfere with respiration and locomotion (Overstreet 1973).
It is concluded the *peritrichous ciliates* are more frequent ciliates causing mortality in cultured shrimps at high density in higher temperature and amonia compounds.

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شیوع و شدت تک یاخته‌های جلدی میگو پا سفید سایت حله، جنوب ایران

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چکیده

توسعه پرورش میگو با بروز بیماری‌های کشنده از جمله ویروسی، باکتریایی و تک یاخته‌ای انگل میگو همراه بوده است. مطالعه ما ارائه وضعیت چند انگل تک یاخته ای مهم میگو پا سفید در استخرهای پرورشی در ایران در طول دوره ۲۰۱۱ تا ۲۰۱۲ شان می‌دهد. به همین منظور یکصد میگو زنده به طور تصادفی ایستگاه حله بوشهر در جنوب ایران تهیه شد. شیوع و شدت دو شاخص مهم است که در این تحقیق بکار گرفته شد. ابتدا از ناحیه آبشش و اسکلت خارجی بدن میگوها نمونه‌های فشاری تهیه گردید و سپس بر روی لام قرار داده شدند. آنگاه لام‌ها جهت بررسی کمی و کیفی در زیر میکروسکوپ مورد بررسی قرار گرفتند. براساس نتایج این مطالعه انگل تک یاخته‌ای *Zoothamnium* SP. بعنوان پاتوژن شایع‌تر در میان مژه داران (۷۹-۸۸/۶٪) در پاهای شنا می‌باشد. در جدایه‌های ما *Acineta* sp. به عنوان عامل شایع در درجه بعدی هم در پاهای شنا و هم در آبشش تائید گردید. لذا اینگونه نتیجه‌گیری می‌شود که، مژه داران مکررا باعث مرگ و میر در میگوهای پرورشی بخصوص در تراکم های بالا ، در درجه حرارت بالاتر و آمونیاک بالا می‌باشد.

واژه‌های کلیدی: میگو، بیماریهای انگلی، مژه دار، شیوع، شدت.

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