Unknown microorganisms in Hemolymph of reared shrimp, *Fenneropenaeus indicus*

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Received: March 2021 Accepted: July 2021

Abstract

Nowadays, Shrimp farmers are suffering from wide spectrum of disease in type of viruses, Bacteria, and parasites. Certain parasites can enforce severe mortalities in shrimp. Among these, protozoa are also very important. The objective of our study was to determine the signs, occurrence, and intensity of unknown microorganisms. The experimental design included three groups, one treatment as infected shrimp or group B and 2 controls with non- infected but exposure to White Spot Syndrom Virus (WSSV) shrimp as group A and non- infected non - exposure to WSSV shrimp as group C in triplicate. The results revealed that unknown-miccroorganisms associated with a decrease in hyalinocytes and large -granulocytes (less than 8%) and a considerable increase in semi-granulocytes in group A and B in contrast to group C that Hyalinocyte increased. This infection made the shrimp susceptible to WSSV disease. It is believed that Hemocytosis causes a condition which can result in a rapid mortality among susceptible species, P. indicus when exposure to WSSV is occurred.

Keywords: Shrimp, *Penaeus indicus*, Unknown microorganism, Intrahemocyte, Mortality

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Introduction

In shrimp, the most significant function of the hemolymph cells are the protection of the shrimp versus invading pathogens including viruses, bacteria, fungus and parasites (Tzou *et al*., 2002; Cerenius and Söderhäll, 2004; Hsieh *et al*., 2008). Researchers reported mortalities in shrimps due to varied pathogenic species such as *Zoothamnium* spp. in *F. indicus* (Kakoolaki, 1997) Microsporidians, cotton or milk shrimp disease (Lightner, 1985), Cephaline gregarine infection with two genera, *Nematopsis* sp. and *Cephalolobus* sp. in *F. indicus* (Rajendran, 1997). An unusual intraerythrocytic protozoa was reported in *Parablennius cornutus* from South Africa (Davies *et al*., 2003). *Haemoproteus* spp. were reported in turtles in Australia, Africa and Asia (Tlford, 2009). In shrimp, the most important role of the circulating hemocytes is the protection of animals against invading microorganisms by participating in recognition, phagocytosis and melanization (Feder, 1999; Fang *et al*., 2004; FAO, 2010). The objective of our study was to describe the signs, prevalence, and intensity of Unknown microorganisms , a new disease in shrimp, *F. indicus.* As it will be discussed, the condition can make the shrimp susceptible to WSSV disease.

Materials and methods

Ten-gram three hundred and seventy live shrimps, *F. indicus* were obtained from a private company in southern part of Iran. Shrimp were transferred to Iran Shrimp Resaerch Center located in Bushehr port. One hundred of shrimp were sampled to collect the hemolymph in order to identify the prevalence and intensity of apicomplexan-like microorganism. Remaining shrimp were distributed to 9 aquariums dimensions $50\times56\times70$ cm^3 as 3 groups, 3 replicates each, A & C, 2 control groups in which the group A included healthy shrimp but exposure to WSSV and group C was known as 2_{nd} control group included infected shrimp with unknown apicomplexan-like microorganisms but not exposure to WSSV. B was the treatment and included infected shrimp with unknown apicomplexan-like microorganisms and exposure to WSSV. The shrimp of groups A & B were then exposed to White Spot Syndrome Virus (WSSV) described in previous study of author (Kakoolaki *et al*., 2011). Group C, as 2_{nd} control shrimp were not exposed to the virus (but with microorganisms). *Hemolymph analysis, Prevalence and intensity of infection* were examined, consequently.

Results

According to our results, some Apicomplexean-like parasite were found in the cytoplasm of hemocytes, granular and semi- granular hemocytes but very few in hyaline cells in *F. indicus* in Groups A and C. No infected cells were observed in group B (Table 1 and Figs. 1 to 5).

Table 1: The prevalence, intensity and DHC of shrimp in various groups.

		Prevalence%	Intensity%	DHC%		
				H*	S*	L-G*
Groups	A	0.00	0.00	5	88	7
	B	93.93	95.63±15.95	8	81	11
	C	84.84	91.50±12.80	15	70	15

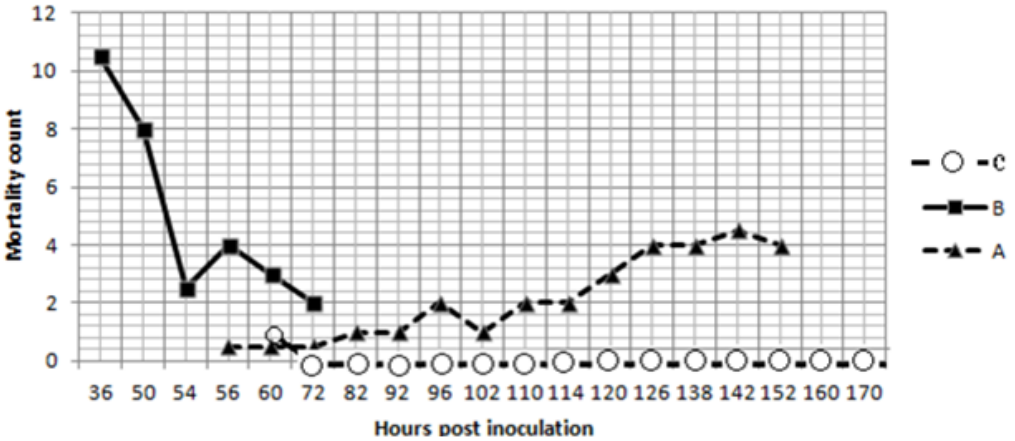

Figure 1: Mortality count among the treatment (B) and controls (A & C) within hpi.

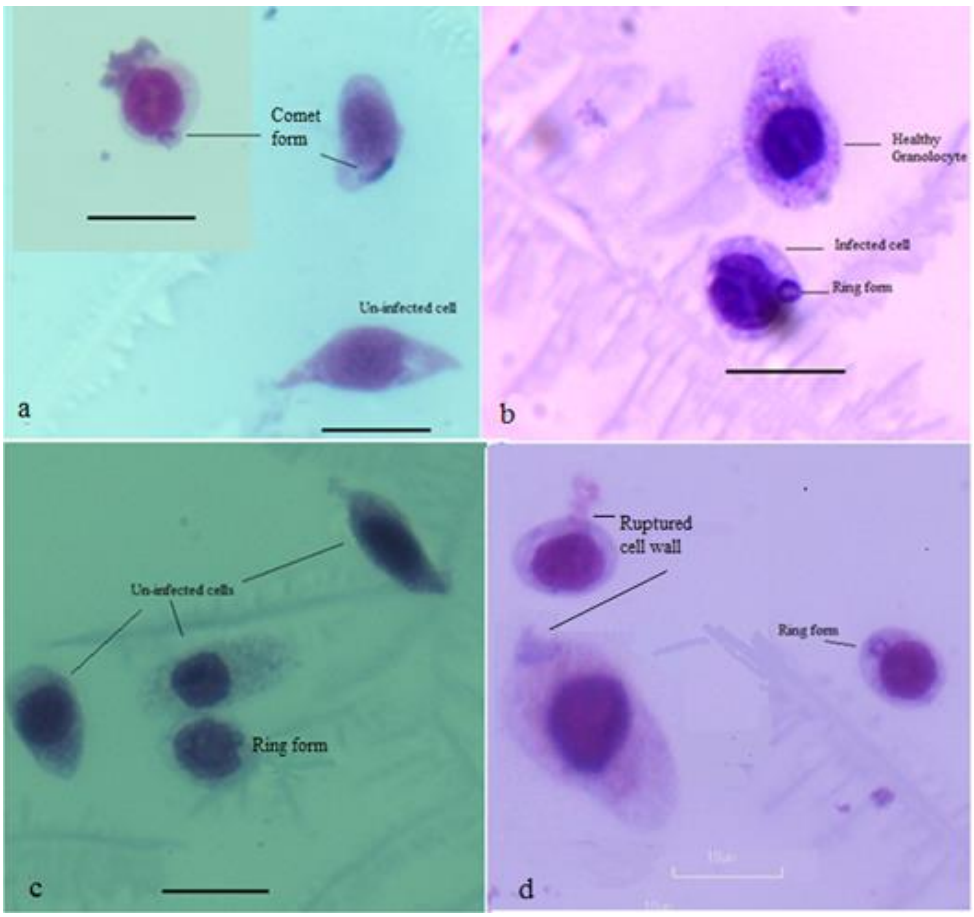

Figure 2: Uninfected and infected shrimp hemocytes with comet and ring forms. Scale bar:10µ × 100, May Grunwald –Giemsa staining.

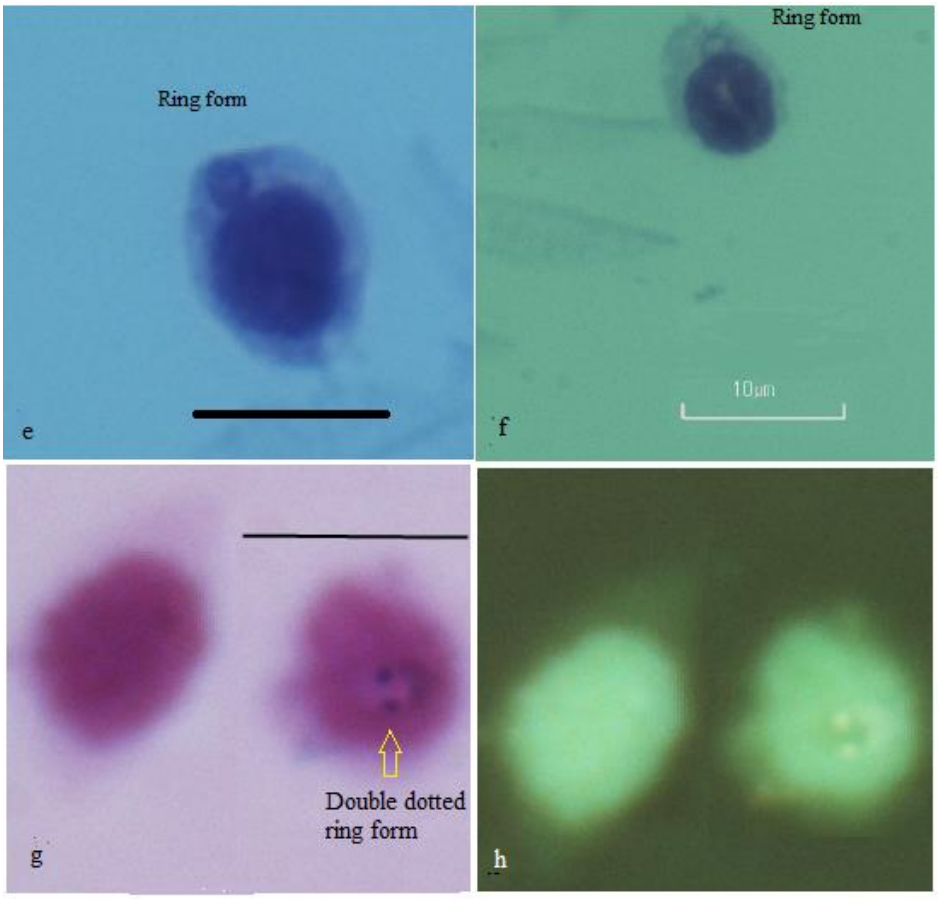

Figure 3: Uninfected and infected shrimp hemocytes × 100, May Grunwald –Giemsa staining.

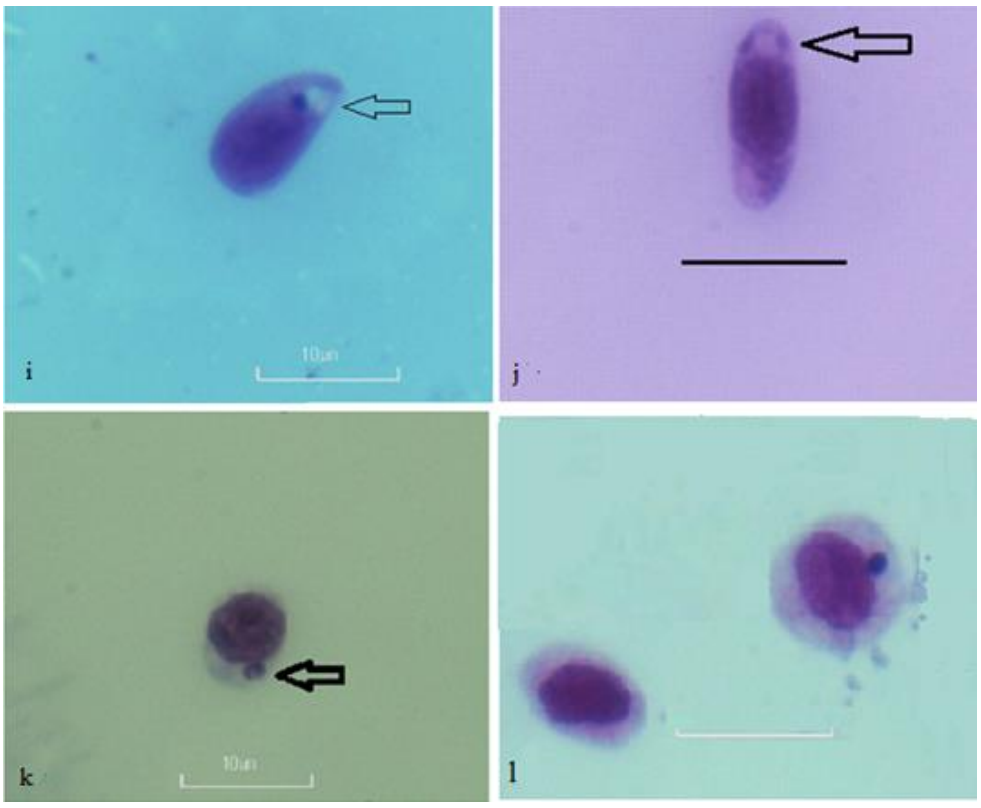

Figure 4: Infected shrimp hemocytes × 100, May Grunwald –Giemsa staining.

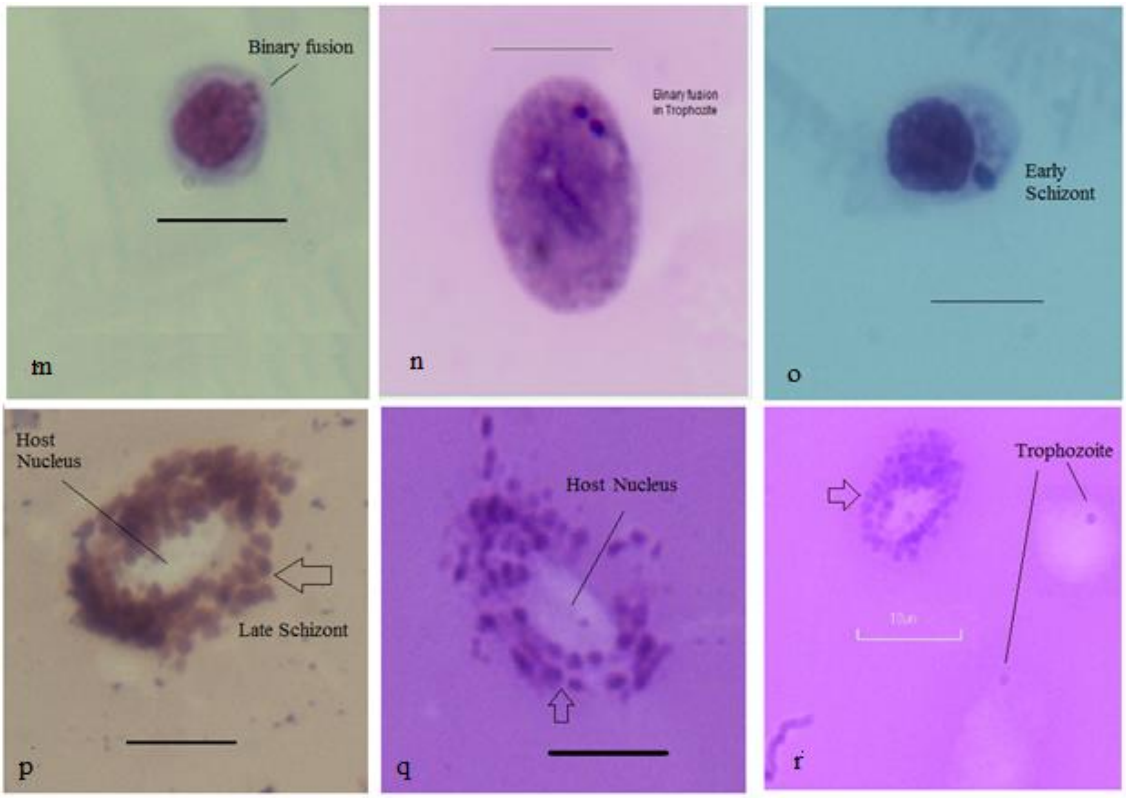

Figure 5: Infected hemocytes at different stages, Scale bar: 10µ.

Discussion

The percent of the HC and LGC of our finding in comparison to the healthy P. indicus (Gopalakrishnan *et al*., 2010; Kakoolaki *et al*., 2010) are considerable less but Semi -granulocytes are more than that of healthy cells. Kondo (2003) believed that semi-granulocytes are the immature LGC. Contrarily, no significant differences between the mean width of these two groups among the mentioned hemocytes were observed. It could be due to more pressure of the microorganisms on the poles of the cells and taking more area.

Using light microscopy, the entire feature of the intrahemocytic unknown microorganism of P. *indicus* similar to those of Apicpmlexean-like parasite similar to the finding of (Nelson, 2008) that showed uni-nucleated stage of the parasite (Haplosporidian- like parasite) with plasmodial forms observes on hemocytes. These forms were visible as early and late throphozoite and schizontlike in colder and warmer weather; respectively conditions indicated that water temperature could be an effective parameter on transitioning stage of the microorganisms. On the other hand, these findings lead us to imagine that small size of the infected hemocytes with Unknown microorganisms could be due to dysfunction and lack of granule production in the cytoplasm because of parasite role. No mortality was observed with Unknown microorganisms group (Group C) dissimilar to the results of Nelson (Hameed *et al*., 2000) who showed that when shrimps are

maintained in captivity the mortality can be high.

The prevalence and intensity of the infection were high and it led us to think about a ubiquitous focal point of the infection in the study area. Severe mortality in group B containing the apicomplexean-like parasites can lead to high stress among the shrimp. However, slow progress of the mortality rate in shrimp of group A and no mortality in group C indicates the enhanced immunity levels in these groups, in comparison to that of group B. Our results indicate that the prevalence and intensity rates of this Apicomplexan-like microorganism are very high. As, the host cells of the parasite belong to immune system of shrimp (Feder, 1999; Cerenius and Söderhäll, 2004; Hsieh *et al*., 2008; FAO, 2010).

It is resulted that Unknown microorganisms did not cause clinical disease, but it was associated with increasing in the amount of Semi-granulocyte and Hyalinocyte rate. It results in declining the level of immunity in susceptible species, *Fenneropenaeus indicus* and makes a rapid mortality among them in exposure to WSSV.

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