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# Silencing a key protein may significantly reduce EHP transmission in farmed shrimp

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## Study results showed the protein EhPTP2 is critical in the life cycle of EHP and that targeting EHP mRNA in host cells can be effective

The microsporidian *Enterocytozoon hepatopenaei* (EHP) infects the hepatopancreas (HP) and intestine of penaeid shrimps including farmed Pacific white shrimp (*Penaeus vannamei*) and black tiger shrimp (*P. monodon*). A severe infection in shrimp leads to retarded growth and a subsequent reduction in productivity.

Microsporidia are obligate intracellular parasites with a characteristic infectious spore that contains an invasion organelle called a polar tube. A double-layered spore wall, consisting of an outer layer (exospore) and a chitin-rich inner layer (endospore), allows the spore to withstand **harsh external environments** (<https://doi.org/10.1128/9781555818227.ch2>).

Upon induction or under opportune conditions, the spore extrudes its polar tube to pierce a host cell in order to initiate an intracellular life cycle in a process called germination. The polar tube serves as a



Recent research has shown that a protein in the polar tube of EHP spores, EhPTP2, plays a crucial role in the life cycle of this important shrimp disease and that dsRNA targeting EHP mRNA can effectively reach the parasite developing in host cells. Therefore, silencing this key protein can significantly reduce EHP transmission in farmed shrimp. This approach is a model for future investigations to identify critical genes for EHP survival and spread as potential targets for preventative and therapeutic measures in shrimp. Photo by Darryl Jory.

conduit through which sporoplasm (contents of a spore of certain parasitic microorganisms, which is injected into and infects a host cell) is transferred into the host cell. Many stimuli have been reported to trigger spore germination as reviewed by **Weiss and colleagues** (<https://doi.org/10.1002/9781118395264.ch10>). The polar tube is made of different polar tube proteins, and to date, seven polar tube proteins have been identified and named **PTP1 to PTP7** (<https://doi.org/10.1016/j.aquaculture.2021.737062>).

Heat inactivation of EHP spores has been demonstrated to prevent spore extrusion, which consequently inhibits the infectivity of the spore, suggesting that the spore extrusion process is a prerequisite for **EHP infection in shrimp** (<https://doi.org/10.1016/j.aquaculture.2020.736178>). RNA interference (RNAi) is a technique that has been applied in various studies of shrimp viruses in both preventative and therapeutic settings. Previous studies support the hypothesis that the silencing of PTPs by RNAi would reduce microsporidian infection.



(<https://link.chtbl.com/aquapod>).

In this study, we hypothesized that the polar tube protein EhPTP2 is associated with spore germination. Therefore, we predicted that silencing of EhPTP2 would inhibit EHP replication, reduce EHP copy numbers, and reduce EHP transmission. This research provides a platform for further development of possible therapeutic or preventive treatments for EHP infection based on the suppression of EhPTP2.

This article – summarized from the **original publication** (<https://doi.org/10.1038/s41598-024-55400-2>). (Yuanlae, S. et al. 2024. Shrimp injection with dsRNA targeting the microsporidian EHP polar tube protein reduces internal and external parasite amplification. *Sci Rep* 14, 4830 (2024) – reports on a study to determine if silencing of EhPTP2 would inhibit EHP replication, reduce EHP copy numbers, and reduce EHP transmission.

## Study setup

To identify proteins involved in the spore germination process, EHP spores were isolated from the hepatopancreas (HP) of EHP-infected *P. vannamei*. Spores were incubated and germinated, and the most abundant EHP spore proteins were selected for analysis.

We hypothesized that the presence of one of these polar tube proteins, EhPTP2, might be required for successful EHP spore extrusion. To test this hypothesis, we injected EhPTP2-specific double-stranded RNA (dsRNA) into healthy shrimp and found that it significantly diminished EHP copy numbers in infected shrimp.

For detailed information on the experimental design and procedures used, refer to the original article.



## Robins McIntosh on everything you need to know about EHP and shrimp farming, part 1

Shrimp farming expert Robins McIntosh details a decade of learning about the microsporidian EHP and how to mitigate its impact.



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## Results and discussion

Our results showed that two methods – Immunofluorescence Assay (IFA; a significant technique commonly used in immunology or molecular biology for detecting the presence and distribution of specific proteins) and Immunohistochemistry (IHC; a form of immunostaining to selectively identify proteins in cells and tissue) – that detect EhPTP2 protein expression can be used to monitor EHP spore germination and localization in infected shrimp.

EHP spore detection through light microscopy is challenging due to the requirement for a high-magnification lens (100x). Targeting specific genes or proteins associated with EHP can offer more sensitive and specific detection. The polar tube protein 2 of EHP (EhPTP2) serves as a valuable target for detection due to its involvement in the polar tube extrusion process, which constitutes a critical step in the parasite's life cycle.

Our research indicates that targeting the EhPTP2 protein can be employed to detect extruded EHP spores in isolated spore samples. This finding suggests that IFA could be beneficial for monitoring EHP spore germination.

Furthermore, beyond the detection of live spores, EhPTP2 can also be utilized as a target for detecting EHP infection in shrimp histological sections using IHC (Fig. 1). Our finding supports the use of EhPTP2 detection as a diagnostic method for EHP infection, which is consistent with previous reports of EhPTP2 detection using other technologies.

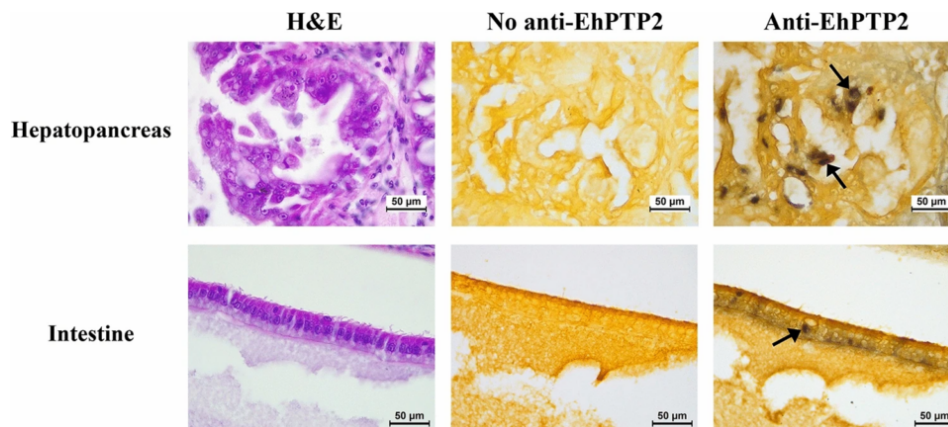


Fig. 1: EHP polar tube protein EhPTP2 detected in the hepatopancreas and intestine using immunohistochemistry. Photomicrographs of 3 consecutive sections of shrimp tissue from a sample collected after 7 days of cohabitation were analyzed by Hematoxylin and Eosin (H&E) staining (left column), negative control immunohistochemistry (middle column), and immunohistochemistry for detecting EhPTP2 using an anti-EhPTP2 antibody (right column). Dark brown precipitates indicate positive signals for EhPTP2 in the hepatopancreatic cells and epithelial cells of the intestine (black arrows). Adapted from the original.

Results also showed that germination in the intestine may not lead to a successful infection. In addition to germinating in the hepatopancreas (HP) as in previous reports, a small number of germinated spores were isolated from the intestine and detected by immunohistochemistry of EhPTP2. Along the digestive tract of shrimp, there is a gastric sieve (GS) which serves as a size-exclusion filter for particles of approximately 0.2–0.7 micrometers in diameter.

Larger particles are diverted to the intestine instead of the HP. Since the average size of EHP oval spores is approximately 1 micron long, it is possible that the germinated spores detected in the intestine were derived from those that were size-excluded from the HP by the GS. While germinated spores and positive signals of EhPTP2 from immunohistochemistry were detected in the intestine, the evidence remains insufficient to conclude that the intestine is another proliferation site of EHP. Instead, we speculate that the presence of the germinated spores in the histology result was the remnant of fecal contamination rather than an active infection.

Regarding the therapeutic and preventative applications of EhPTP2 suppression, as EhPTP2 is associated with polar tube extrusion, it suggests that suppression of EhPTP2 messenger ribonucleic acid (mRNA; involved in protein synthesis in cells) would inhibit the extrusion process and result in reduced EHP infection. This was indeed the case when EhPTP2-specific double-stranded ribonucleic acid (dsRNA; associated with most viral infections, it either constitutes the viral genome in the case of dsRNA viruses or is generated in host cells during viral replication) was intramuscularly injected into previously EHP-infected shrimp (Fig. 2).

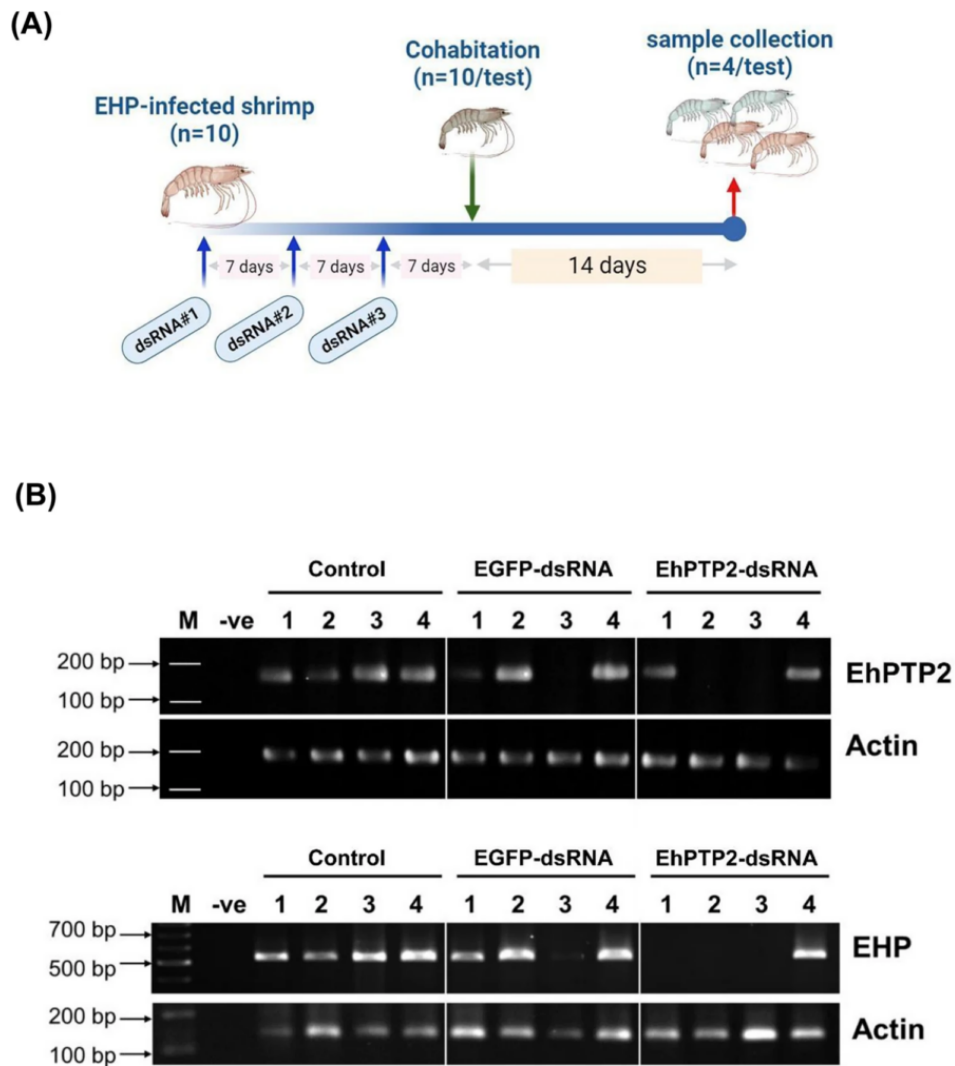


Fig. 2: Suppression of EhPTP2 mRNA expression in EHP-infected shrimp negatively affects the horizontal transmission rate. (A) A diagram representing the experimental design to test how EhPTP2 dsRNA affected horizontal EHP transmission to naïve shrimp. The EHP-infected shrimp were injected with three doses of dsRNA, at three 7-day intervals, and then the naïve shrimp were cohabitated for 14 days. (B, upper panel) RT-PCR detection of EhPTP2 transcript expression in naïve shrimp post-cohabitation and PCR detection of SWP gene accumulation also indicated a low replication rate of EHP in naïve shrimp when cohabitated with EHP-infected shrimp that were previously injected with EhPTP2dsRNA (lower). M = DNA marker, - ve = negative control. Adapted from the original.

The inhibition of infection when the RNAi technique is applied preventatively is not uncommon when key virulent genes are knocked down in naïve shrimp prior to **pathogen exposure** (<https://doi.org/10.1016/j.jbiotec.2016.05.007>). For example, in one cultured pond, high variation of EHP infection levels in the population was observed. The 25 percent of shrimp injected with dsRNA and showed EHP infection might be those infected with high levels of EHP. If the fourth dose of dsRNA-

EhPTP2 can be applied, it might be able to reduce the infection. What was promising, in this study, is the fact that administration of EhPTP2-specific dsRNA could be utilized therapeutically once infection had already been established in two aspects: (1) reducing EHP loads in infected shrimp and (2) reducing further EHP spread in shrimp ponds. Only a handful of reports have documented successful therapeutic applications in **pathogen-infected shrimp** (<https://doi.org/10.1016/j.virusres.2010.09.009>).

Considering that EHP infection is chronic and that the negative impact on growth is not noticeable until the EHP copy number reaches a critical threshold of  $10^3$  copies per nanogram of total hepatopancreas DNA, available data suggest that the combination of routine surveillance and therapeutic application of dsRNA against EHP polar tube protein EhPTP2 may have potential for controlling EHP.

## Perspectives

The results of this study revealed that EhPTP2 plays a crucial role in the life cycle of EHP and that dsRNA targeting EHP mRNA can effectively reach the parasite developing in host cells. This approach is a model for future investigations to identify critical genes for EHP survival and spread as potential targets for preventive and therapeutic measures in shrimp.

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