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Health & Welfare

Frequent urination can be hazardous to health of farmed shrimp

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Study fully describes, renames shrimp excretory organ as the nephrocomplex and identifies it as major portal for pathogens

Results of this study show that the nephrocomplex, previously known as the antennal gland, is much more complex than previously assumed. Its anatomy, morphology and cellular structure are optimal for the excretion organ to be a pathogen entry portal. Results also showed links to the molting process. Overall, the findings will help accelerate the growth of shrimp and protect them against pathogens. Photo by Fernando Huerta.

The White Spot Syndrome Virus (WSSV) is considered a major cause of production losses for the crustacean aquaculture industry, and its impact is estimated at 10 percent or several hundred million U.S. dollars annually. This virus is of particular concern to leading scientists because it hinders the future global food supply, as aquaculture is one of the most important food sources to meet the increasing demand of a growing global population. Viruses are not the only pathogens that cause serious damage to the global farmed shrimp industry globally, as bacterial infections like vibriosis result in an estimated 20 percent production loss annually.

Researchers have proposed various routes for the transmission of WSSV but there is still considerable debate on the exact portal of entry. Considering the question of how WSSV (and other pathogens such as *Vibrio*) can enter the shrimp body and what shrimp structures are connected to the outside world but are not lined with cuticle [tough, flexible, outer coverings of an organism or parts of an organism that give it protection], we investigated the potential of the antennal gland as a portal of entry for pathogens.

The antennal gland has been reported to be among the very first organs to become infected, and previous research has shown some pathogens present in the antennal gland of crustaceans. However, to date no evidence has been presented that the antennal gland can be an entry portal for pathogens and can act as a primary replication site.

This article – adapted and summarized from the **original publication** (http://www.pnas.org/cgi/doi/10.1073/pnas.2013518117) (De Gryse, G.M.A. et al. 2020. The shrimp nephrocomplex serves as a major portal of pathogen entry and is involved in the molting process) – reports on an investigation of the role of the antennal gland as a possible entry route and primary replication site of WSSV and *Vibrio* species.

For detailed information on the study setup, refer to the original publication.

Results and discussion

In this study, we fully explored the anatomy of the antennal gland. Our results showed that this organ has a substantially wider distribution throughout the cephalothorax [fused head and thorax of shrimp] and is a much more complex structure than previously assumed. Our research showed that the antennal gland is a perfect portal for pathogen entry and that its wide distribution in the shrimp cephalothorax results in close contact to all WSSV-susceptible organs: the nervous system, the alimentary tract, the lymphoid organ, the gills, the hepatopancreas and various muscles. Only the heart is not in close contact with the antennal gland.

The antennal gland's structure with no cuticular lining allows for the potential rapid entry of pathogens into the shrimp hemolymph [shrimp fluid analogous to the blood in vertebrates]. Because of the vast distribution of the organ in the cephalothorax and the antennal gland's function as an excretory organ, among other reasons, we propose a new name for this organ: the nephrocomplex. The prefix "nephron" is from the Greek "nephros," for kidney, and the suffix "complex" is because of the large numbers of diverse subunits in this excretory organ. Furthermore, it seems that the filling of certain parts of the nephrocomplex is linked to the molting process and could potentially play crucial mechanical roles in the process.

We confirmed our hypothesis of the nephrocomplex being a major pathogen entry portal candidate by intrabladder inoculation of shrimp with WSSV and *Vibrio campbellii*, leading to both morbidity and mortality. Compared to intramuscular inoculation, only 56 times more infectious WSSV is needed to infect shrimp via intrabladder inoculation, while 28.8×10^6 times more virus is necessary to infect shrimp via oral inoculation. For *Vibrio*, similar results were obtained (62 and $>10^9$ times more, respectively).

These data show that the intrabladder infection is almost as efficient as the intramuscular inoculation, which bypasses all natural defense barriers. In our pathogenesis experiment, we clearly demonstrated that, when infected via the nephropore [opening of the nephrocomplex on ventral surface of shrimp], WSSV first replicates in the bladder and then spreads all over the body. This proves the possibility and efficiency of viral spread from the tissues of the nephrocomplex into the hemocoel [cavity or series of spaces between the organs of most arthropods and mollusks through which the blood or hemolymph circulates], where the virus can infect all other susceptible organs.

A drop in salinity has been proven to facilitate WSSV infection. We found that, during such conditions, shrimp urine tested positive for WSSV before hemolymph. After 24 hours post-inoculation (hpi) and beyond, the presence of increasing amounts of WSSV copies further supports the nephrocomplex's role as primary replication site. However, to provide strong evidence that the nephrocomplex functions as a natural portal of entry, proof of pathogen entry was required.

We tested the integrity of this nephropore barrier with an experimental ex vivo setup, where pressure from inside of the shrimp (simulated urination) allowed the valves to open, whereas pressure from outside of the shrimp did not cause the valve seal to be compromised. Under normal conditions, the

only time this cuticular valve opens is during urination. Specific conditions during which the nephropore opens more often could provide pathogens with a window of opportunity for pathogen invasion.

A sudden drop in salinity, resulting in frequent urination (and thus frequent opening of the nephropore), is such a condition. Because of the nephrocomplex's role in regulation of hemocoel volume, the sudden decrease in salinity prompts the shrimp to produce and expel urine in higher quantities and at higher frequency. This and other findings from our research together provide strong evidence for the nephrocomplex being a major portal of pathogen entry.

Perspectives

Our study results show that the nephrocomplex, previously known as the antennal gland, is much more complex than previously assumed. Its anatomy, morphology and cellular structure are optimal for the excretion organ to be a pathogen entry portal. Also, links to the molting process were found using magnetic resonance microscopy (μMRI), a microscopic imaging technology down to the scale of microns.

Additionally, the sealing function of the nephropore valves was examined and found to be an efficient pathogen barrier. However, it was demonstrated that, at the end of the urination process, this function is briefly compromised. Thus, during conditions where frequent urination takes place (sudden salinity drop during, e.g., heavy monsoon rains, aggression, establishment of social dominance, feed intake and possibly after molting) combined with a high virus load in the surrounding water, the nephrocomplex has to be considered a major portal of pathogen entry.

The findings of our study will cause a major shift in shrimp pathogen research, especially in the field of WSSV where all current findings are, until now, solely based on intramuscular and peroral inoculations. WSSV pathogenesis and immunity studies have to be performed using intrabladder inoculation or via immersion upon drop in salinity. Also, the identification of the nephrocomplex as an entry portal will focus the search for control measures to this organ. It will also allow for a direct breeding program for pathogen resistance.

Finally, it confirms the empirical observation of shrimp farmers that periods of heavy rainfall are linked to major outbreaks of WSSV infections in open-air ponds.

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