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Vaccinating Nile tilapia against Tilapia Lake Virus (TiLV)

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Results show that immunization of tilapia broodstock with TiLV vaccines could be a potential strategy for the prevention of this important disease

Results of this study showed that vaccination of tilapia broodstock with a heat-killed TiLV vaccine (HKV) or a formalin-killed TiLV vaccine (FKV) produces a protective antibody response against TiLV, and that these antibodies can be transferred to the fertilized eggs and larvae to induce maternal immunity. Photo by Darryl Jory.

Tilapias are one of the most important freshwater fish species farmed globally and are now cultured in more than 140 countries. The Tilapia Lake Virus (TiLV), also known as *Tilapia tilapinevirus*, is one of the most significant infectious agents causing relatively high mortality and economic losses for tilapia farmers. The mortality rate in natural TiLV outbreaks ranges from 20 to 90 percent, while cumulative mortalities from experimental infection range from 66 to 100 percent. The virus can infect fertilized eggs, yolk-sac fish, fry, fingerlings and adult fish. Recent studies have reported that TiLV can also be transmitted vertically from infected broodstock to their offspring.

Vaccination is an effective strategy to prevent infectious diseases in aquaculture, and several vaccines have been described for the control of TiLV in tilapia. Most recently, water-based inactivated vaccines prepared with heat-killed or formalin-killed TiLV were shown to provide good levels of protection in juvenile tilapia. It has also been reported that TiLV vaccines can induce both humoral [through antibodies] immunity and cell-mediated [without antibodies] immunity.

This article – adapted and summarized from the **original publication** (https://doi.org/10.3390/vaccines10020167) [Mai, T.T. et al. 2022. Immunization of Nile Tilapia (*Oreochromis niloticus*) Broodstock with Tilapia Lake Virus (TiLV) Inactivated Vaccines Elicits Protective Antibody and Passive Maternal Antibody Transfer. *Vaccines* 2022, 10(2), 167] – reports on a study to investigate levels of TiLV-specific antibodies in Nile tilapia broodstock after immunization with either heat-killed vaccine (HKV) or formalin-killed vaccine (FKV), and the role of these antibodies in protecting juvenile tilapia against TiLV through passive immunization. We also assessed the transfer of maternal antibodies from vaccinated broodstock to their fertilized eggs and larvae.

Study setup

We hypothesized that Nile tilapia (*Oreochromis niloticus*) broodstock immunized with a TiLV inactivated vaccine can mount a protective antibody response and passively transfer maternal antibodies to their fertilized eggs and larvae. To test this hypothesis, three groups of tilapia broodstock, each containing four males and eight females, were immunized with either a heat-killed TiLV vaccine (HKV), a formalin-killed TiLV vaccine (FKV).

Booster vaccination with the same vaccines was given three weeks later, and mating took place one week thereafter. Broodstock blood sera, fertilized eggs and larvae were collected from six to 14 weeks post-primary vaccination for measurement of TiLV-specific antibody (anti-TiLV IgM) levels. In parallel, passive immunization using sera from the immunized female broodstock was administered to naïve [not previously exposed to the virus] tilapia juveniles to assess if antibodies induced in immunized broodstock were protective.

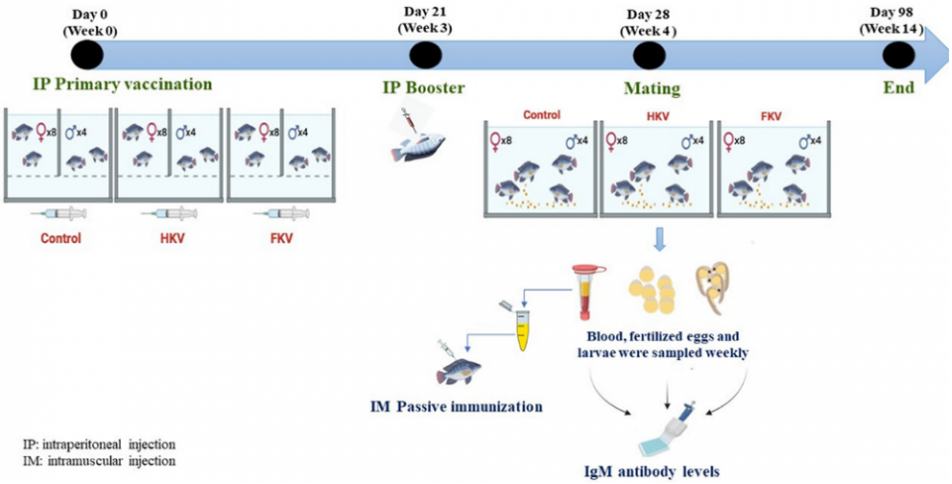

Fig. 1: Diagram illustrating the experimental design for broodstock TiLV vaccination, mating and sampling.

For detailed information on the experimental design and fish; animal husbandry; vaccine preparation and dosages; immunization, breeding and sampling, and other topics in this study, refer to the original publication.

Results and discussion

In a **previous and related study** (https://doi.org/10.1111/jfd.13523), vaccination of tilapia juveniles with HKV and FKV resulted in a significant increase in systemic TiLV-specific IgM and a high level of protection against TiLV challenge (relative percent survival, RPS = 71.3 to 79.6 percent). However, the persistence of a specific antibody was not evaluated.

In our current study, we used the same vaccination protocol for the tilapia broodstock, using double doses of antigen per fish as in our previous study, for both primary immunization and the booster vaccination. Relatively high levels of TiLV-IgM were detected from six to 14 weeks post-primary vaccination, suggesting that both HKV and FKV elicited relatively long persistence (98 days) of TiLV-IgM in vaccinated broodstock. This finding is consistent with a previous observation in tilapia juveniles challenged with TiLV, where a specific antibody response was maintained for six to 16 weeks post-infection.

Although the protective efficacy of several TiLV vaccines has been reported recently, the specific role of anti-TiLV antibodies against TiLV challenge is still unclear, since several studies have reported that TiLV vaccines can stimulate both humoral immunity and cell-mediated immunity. In our study, the high survival of passive immunized tilapia (85 to 90 percent) after receiving sera from the vaccinated broodstock (both HKV and FKV), suggests that humoral immunity plays an important role in protecting against TiLV infection through anti-TiLV antibodies.

The reduction in TiLV load during the course of infection, which decreased to undetectable levels in surviving fish by the end of the experiment, reinforces the presumed role of protective antibodies in virus clearance. Theoretically, these antibodies could be capable of removing TiLV from the body of the fish by various mechanisms, and several studies have shown that passive immunization can protect fish from viral infection. Because tilapia broodstock are usually kept in the hatchery for three to five years, vaccination would be an effective strategy to prevent TiLV infection in the broodstock, minimizing economic loss and maintaining good health of the broodstock during the breeding period.

Our results provide evidence that maternal antibodies from TiLV-vaccinated tilapia broodstock are transferred to their offspring. Interestingly, these antibodies were found to be protective during passive immunization in tilapia juveniles challenged with the virus. This suggests that anti-TiLV antibodies may not only help to reduce the risk of infection in broodstock but may also reduce the risk of vertical TiLV transmission. Several studies have reported

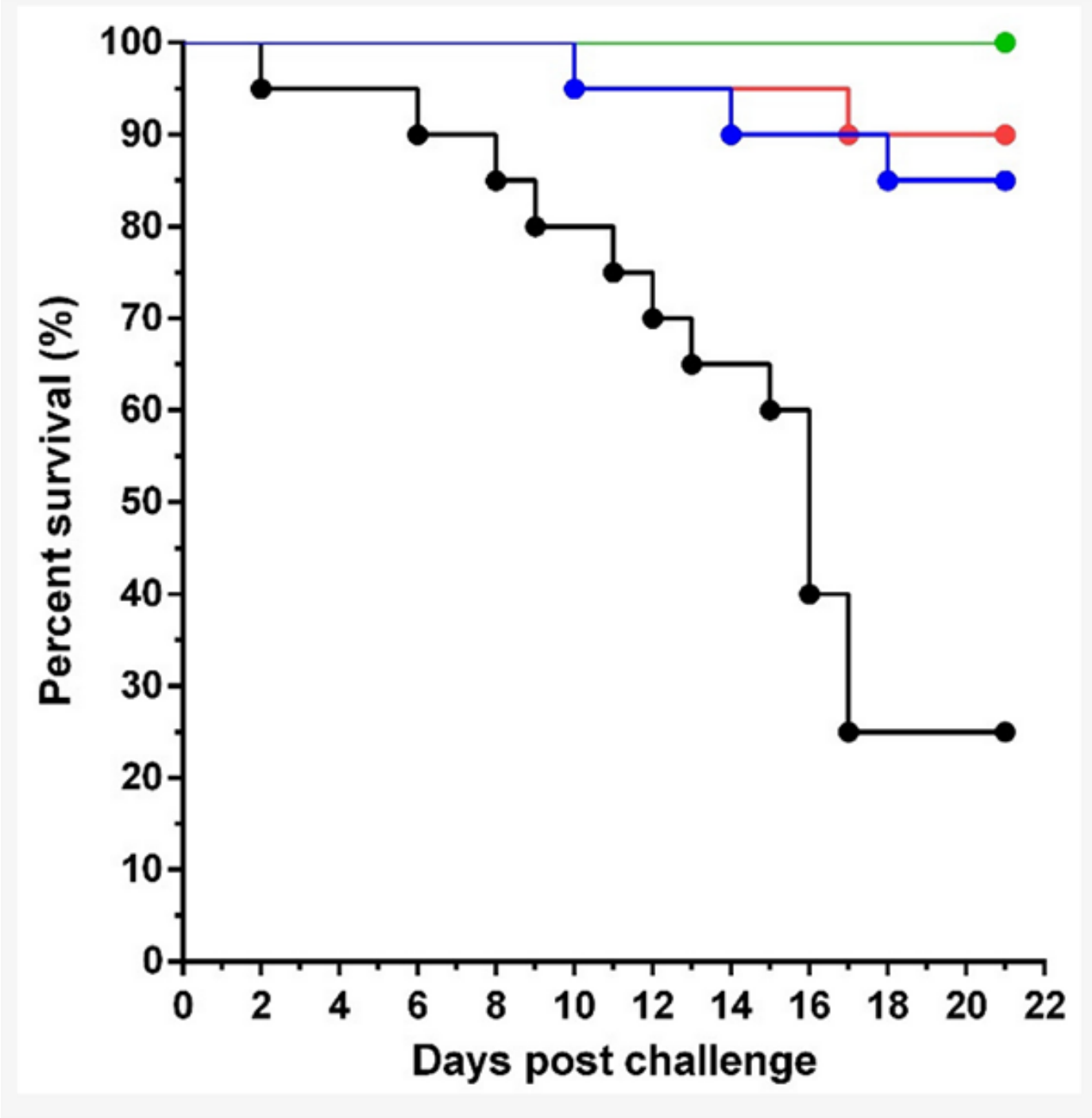

Fig. 2: Average percent survival of Nile tilapia juveniles passively immunized with pooled sera from female broodstock by intramuscular injection (IM) and then challenged with TiLV TH-2018 at 9 × 10^5 TCID50 per fish [The 50 percent Tissue Culture Infectious Dose assay, or TCID50, is a traditional method used by virologists to determine viral titers in both stocks and samples]. The differences were statically significant between groups 1, 2, 4 and group 3 (n = 20 per group). HKV, FKV, and control mean broodstock fish were immunized with a heat-killed vaccine, formalin-killed vaccine and L15 medium, respectively. The L15 group is a negative control group treated with L15 medium without virus (n = 20). Adapted from the original.

that vaccination of broodstock is an effective strategy to enhance the maternal transfer of immunity from mother

to offspring and reduce the risk of vertical transmission of the pathogen.

Higher levels of TiLV-IgM were found in the fertilized eggs of the group vaccinated with HKV than in those of the fish vaccinated with FKV, suggesting that HKV is more promising for successful maternal vaccination. However, TiLV-IgM transfer only persisted for one to three days post-hatch and was undetectable by seven and 14 days post-hatch. Because the TiLV challenge was unsuccessful at the larval stage of tilapia, we did not evaluate passive antibody protection in the offspring. However, these findings suggest that maternal antibody transfer in larvae does not last long and may be insufficient to protect offspring after one to three days post-hatch.

This result agrees with the results observed in other fish species like grouper vaccinated against Nervous Necrosis Virus, NNV, where specific antibodies were found to gradually decrease within 48 hours after hatching. Such short persistence can be explained by the gradual decline in IgM during yolk-sac absorption observed in tilapia, and other fish such as European sea bass (*Dicentrarchus labrax*) and Atlantic salmon (*Salmo salar*). Therefore, in addition to vaccination, biosecurity measures remain essential to prevent the introduction of pathogens into tilapia hatcheries, especially during seed production.

Perspectives

Results of our study have shown that vaccination of tilapia broodstock with HKV and FKV elicits a protective antibody response against TiLV, and that these antibodies can be transferred to the fertilized eggs and larvae to induce maternal immunity. HKV appears to have greater potential than FKV for maternal transmission of antibodies. However, protective antibodies had a short persistence in the larvae leaving a gap between maternal immunity and immunocompetence. Further vaccination is therefore likely to be needed to protect fish from TiLV infection during this gap, as well as for later stages of development.

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