ORIGINAL ARTICLE

Modelling the effect of vaccination on transmission dynamics of nervous necrosis virus in grouper larvae *Epinephelus coioides*

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Abstract

Nervous necrosis virus (NNV) infection in susceptible grouper larvae has been reported to cause high mortalities, leading to great economic losses in aquaculture industry. Although the effects of NNV vaccines on grouper have been broadly investigated, vaccination strategies have not been fully established. To this end, we introduced the parsimonious epidemiological models that explored the assessment of key epidemiological parameters and how they changed when vaccinations showed the effects. We showed that the models capture the published cumulative mortality data accurately. We estimated a basic reproduction number $R_0 = 2.44$ for NNV transmission in grouper larvae without vaccination. To effectively control NNV transmission by vaccination, a model for disease control was also generalized to attain the goals of controlled reproduction number less than 1. Our results indicated that at least 60% of grouper population needed to be immunized for ~75 min. Our data-driven modelling approach that links the transmission dynamics of NNV and vaccination strategies for grouper has the potential to support evidence-based planning and adaptation of integrated control measures. We encourage that the epidemiology-based framework introduced here can be further implemented for establishing effective vaccination and mitigation actions aimed at controlling diseases in fish farming practices.

KEYWORDS
control measure, grouper, mathematical modelling, nervous necrosis virus, vaccination

1 | INTRODUCTION

Betanodaviruses, also referred to as the nervous necrosis viruses (NNVs), have infected more than 50 species of marine and freshwater fish (Chi, Wu, & Hong, 2016; ICTV, 2019). The disease caused by NNV is called viral nervous necrosis (VNN) disease and is also known as viral encephalopathy and retinopathy (VER) (Chi et al., 2016). VNN disease could spread in fish population via horizontal and vertical transmissions and be reproduced in healthy fish by cohabitation and immersion (Arimoto, Mori, Nakai, Muroga, & Furusawa, 1993; Kai & Chi, 2008; Mori et al., 1991). As the major pathogen with a high impact on the aquaculture industry, NNV has imposed a serious threat to the production of grouper larvae in Taiwan where the production

of grouper is the second worldwide (Chi et al., 2016; FRI, 2018; ICTV, 2019; Yeh, Chu, & Chen, 2017).

To mitigate the impacts of VNN diseases on the production of grouper larvae, farmers need to adopt suitable and effective control strategies. Although researchers have dedicated to investigate the effects of NNV vaccines on cumulative mortality in grouper larvae, vaccination strategies have not been established in farmed ponds (Kai & Chi, 2008; Kai, Wu, & Chi, 2014). Epidemiological modelling which has been broadly used to study infectious diseases in terrestrial systems not only could help to understand the effects of key factors on the disease dynamics but also could be applied to inform critical information for the development of disease control policies in aquatic environment (Munang'andu, 2016; Ögüt, 2001;

Peeler & Taylor, 2011; Reno, 1998; Thrush, Murray, Brun, Wallace, & Peeler, 2011). The transmission dynamic models could be used to assess various epidemiological parameters and the population-level epidemic dynamics as well as to assess the impact of vaccination on the spread of disease (Keeling & Rohani, 2008; MacLachlan & Dubovi, 2016; Rao & Kumar, 2015).

A basic susceptible–infected–recovered (SIR) model has been widely used to describe the infectious disease epidemics in aquatic animals (Alaliyat & Yndestad, 2015; Krkošek, 2010; Murray, 2008, 2009; Ögüt, 2001; Ögüt, LaPatra, & Reno, 2005). Based on the characteristics of the progress of infectious diseases in aquatic species, other health status of host population such as exposed and latent compartments was incorporated into the basic SIR model (Aldrin, Huseby, & Jansen, 2015; Lotz & Soto, 2002; Salama & Murray, 2011). However, relative data of fish or other marine species are often unavailable because of the gap between the theoretical models and the experiment designs. Therefore, a SIR-based susceptible–infectious–mortality (SIM) model was developed specially for aquaculture species to explore the population dynamics of disease transmission in a closed fish population (Liao et al., 2006).

Vaccination is one of the most effective interventions and innovative strategies for research, and development of vaccines must be encouraged in aquaculture (Kai et al., 2014; MacLachlan & Dubovi, 2016). Although there is an effort to develop vaccines for grouper against NNV in Taiwan, commercial application is still not established due partly to the lack of a cheaper manufacturing process and effective protection (Lin, Jiang, Chen, Wang, & Chen, 2018). Hence, to provide insight into the development of NNV vaccination programme, we tended to apply the transmission dynamic models to evaluate the effectiveness of vaccination. To achieve the purpose, firstly, we constructed the SIM-based transmission dynamic models to describe the population dynamics of grouper larvae infected with NNV in the scenario of with or without vaccination. Secondly, epidemiological parameters were estimated and their sensitivities to cumulative mortality were also performed. Lastly, we developed a vaccination-based model for disease control to provide implications for effectively containing the spread of NNV in grouper larvae.

2 | MATERIALS AND METHODS

2.1 | Study data

To explore the population dynamics of grouper larvae infected with NNV in the scenarios of with or without vaccination, cumulative mortality data were acquired from the published NNV exposure experiments (Figure S1). Kai and Chi (2008) conducted several NNV exposure trials to test the efficacies of applying formalin- and binary ethylenimine (BEI)-inactivated vaccines in grouper larvae. In their experiments, grouper larvae *Epinephelus coioides* (*E. coioides*) obtained from a hatchery farm in southern Taiwan were kept in a 400-L tank, whereas NNV isolated from a humpback grouper *Cromileptes altivelis* was used for preparation of inactivated vaccines.

Briefly, among inactivated vaccines, BEI-inactivated vaccines showed the best efficacy. Subsequently, further NNV exposure trials were designed to assess the efficacies of BEI-inactivated vaccines on grouper larvae that were bath-immunized for different immersion times. In each group, 100 grouper larvae were reared at 24–27°C in a 60-L aquarium prior to bath immunization. The 40-day-old grouper larvae in the mock vaccine trial (i.e. control group) were mock-immunized with phosphate buffer (PBS), whereas those in the NNV vaccine groups (i.e. the vaccine group) were bath-immunized with BEI-inactivated vaccines for 20, 60 and 120 min, respectively (Figure S1). After 30 days, each group was immersed with NNV at concentration of 1.6×10^6 50% tissue cultured-infected dose (TCID50) mL^{-1}. Cumulative mortality was recorded for 30 days post-NNV immersion (Figure S1 and Table S1).

In this study, the time-course cumulative mortality data obtained from the control and vaccine groups were applied to estimate epidemiological parameters of NNV transmission in grouper larvae population. Effectiveness of vaccines in different treatment durations was also evaluated.

2.2 | Transmission dynamic model

Nervous necrosis viruses that invades the host by rapidly reaching the central nervous tissues may induce death or remain for several years in survivors (OIE, 2018). NNV-infected larvae were likely to survive, acting as carriers for the next generation (Kuo, Wang, Hsu, Lee, et al., 2012; Nerland, Skaar, Eriksen, & Bleie, 2007). Moreover, persistently NNV-infected fish had high potential to transmit infection to other health fish (Johansen, Ranheim, Hansen, Taksdal, & Totland, 2002). Once they become a carrier, the spread of NNV would be facilitated.

Based on the characteristics of NNV transmission in grouper larvae, the carrier and immunity compartments can be incorporated into the SIM model to describe the transmission dynamics in host population without vaccination (i.e. the control group) and are designated as the susceptible–infectious–mortality–carrier–natural immunity (SIMCI$_N$) model as Equations T1–T5 (Table 1 and Figure 1a) where $S(t)$, $I(t)$, $M(t)$, $C(t)$ and $I_N(t)$ represent the proportion of grouper larvae in susceptible, infectious, mortality, carrier and natural immunity states at time t, k is the generation rate of initial infectious population (day^{-1}), τ is the time for initial infectious population appearance (day), β_I is the transmission rate of infectious (day^{-1}), β_C is the transmission rate of carriers (day^{-1}), γ_I is the rate of individuals leaving infectious population (day^{-1}), q is the proportion of infectious that become carriers (–), and γ_C is the rate of individuals leaving carrier population (day^{-1}). Since there were no infectious population initially, it took time for experimental grouper larvae that were susceptible to generate initial infectious population. Therefore, the Dirac delta function $\delta(t-\tau)$ defined as $\delta(t-\tau) = 0$, $t \neq \tau$ and $\int_{-\infty}^{\infty} \delta(t-\tau)\,dt = 1$ representing the unit impulse function which is zero for all t except $t = \tau$ is used in the term of $k \times \delta(t - \tau)$ to capture that k is suddenly inputted into this system at τ.

TABLE 1 Equations for susceptible–infectious–mortality–carrier–natural immunity (SIMCI$_N$), vaccination-based susceptible–infectious–mortality–carrier–natural/vaccinated immunity (SIMCI$_{NV}$), and disease control models used in this study (see text for the symbol meanings)

Equations
Susceptible–infectious–mortality–carrier–immunity (SIMCI$_N$) model
$\frac{dS(t)}{dt} = -k\delta(t-\tau) - \beta_I S(t) I(t) - \beta_C S(t) C(t)$ (T1)
$\frac{dI(t)}{dt} = k\delta(t-\tau) + \beta_I S(t) I(t) + \beta_C S(t) C(t) - \gamma_I I(t)$ (T2)
$\frac{dM(t)}{dt} = (1-q)\gamma_I I(t)$ (T3)
$\frac{dC(t)}{dt} = q\gamma_I I(t) - \gamma_C C(t)$ (T4)
$\frac{dI_N(t)}{dt} = \gamma_C C(t)$ (T5)
Vaccination-based SIMCI$_{NV}$ model
$\frac{dS(t)}{dt} = -k_V\delta(t-\tau_V) - (1-p)\beta_I S(t) I(t) - (1-p)\beta_C S(t) C(t)$ (T6)
$\frac{dI(t)}{dt} = k_V\delta(t-\tau_V) + (1-p)\beta_I S(t) I(t) + (1-p)\beta_C S(t) C(t) - \gamma_I I(t)$ (T7)
$\frac{dM(t)}{dt} = (1-q)\gamma_I I(t)$ (T8)
$\frac{dC(t)}{dt} = q\gamma_I I(t) - \gamma_C C(t)$ (T9)
$\frac{dI_{NV}(t)}{dt} = \gamma_C C(t)$ (T10)
Disease control model
$R_C = R_0(1-p_b p) = R_0(1-p_b f(t_b))$ (T11)

FIGURE 1 Schematic of the NNV transmission dynamic models describing population dynamics of grouper larvae exposed to NNV (a) without: the SIMCI$_N$ model and (b) with vaccination: the vaccination-based SIMCI$_{NV}$ model. The S, I, M, C, I$_N$ and I$_{NV}$ represent the states in which the grouper larvae are susceptible, infectious, carrier, natural immunity and natural/vaccinated immunity, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

The epidemiological parameters in the SIMCI$_N$ model (β_I, β_C, γ_I, q, γ_C) can be estimated by fitting Equations T1–T5 to the cumulative mortality data of grouper larvae exposed to NNV without vaccination with initial condition {$S(0)$, $I(0)$, $M(0)$, $C(0)$, $I_N(0) = 1, 0, 0, 0, 0$}.
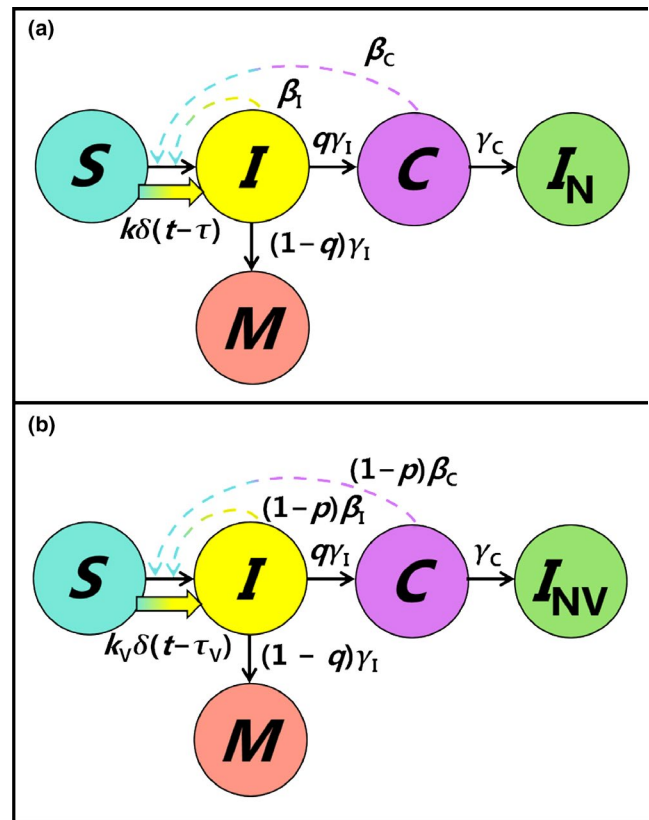
The basic reproduction number (R_0), defined as the average number of secondary cases generated by a primary case during entire infectious period in an entirely susceptible population, is a measure of the potential for disease outbreaks. $R_0 < 1$ indicates that a disease will disappear over time, whereas $R_0 > 1$ means that there will be an epidemic (Anderson & May, 1991; Keeling & Rohani, 2008). Based on the next-generation method (van den Driessche & Watmough, 2002), R_0 for NNV transmission in grouper larvae could be calculated as.

In the SIMCI$_{NV}$ model, $I_{NV}(t)$ represents the proportion of grouper larvae in natural/vaccinated immunity states at time t, p is the proportion of successful immunization, and k_V and τ_V are the generation rate of initial infectious population (day^{-1}) and the time for initial infectious population appearance (day) for vaccinated grou-

$$R_0 = \rho\, FV_t^{-1} = \frac{\beta_I}{\gamma_I} + \frac{q\beta_C}{\gamma_C}, \quad F = \begin{bmatrix} \beta_I & \beta_C \\ 0 & 0 \end{bmatrix}, \quad V_t = \begin{bmatrix} \gamma_I & 0 \\ -q\gamma_I & \gamma_C \end{bmatrix}, \quad V_t^{-1} = \frac{1}{\gamma_C \gamma_I}\begin{bmatrix} \gamma_C & 0 \\ q\gamma_I & \gamma_I \end{bmatrix} = \begin{bmatrix} \frac{1}{\gamma_I} & 0 \\ \frac{q}{\gamma_C} & \gamma_C \end{bmatrix} \quad \text{and} \quad FV_t^{-1} = \begin{bmatrix} \beta_I & \beta_C \\ 0 & 0 \end{bmatrix}\begin{bmatrix} \frac{1}{\gamma_I} & 0 \\ \frac{q}{\gamma_C} & \frac{1}{\gamma_C} \end{bmatrix} = \begin{bmatrix} \frac{\beta_I}{\gamma_I} + \frac{q\beta_C}{\gamma_C} & \frac{\beta_C}{\gamma_C} \\ 0 & 0 \end{bmatrix},$$

where F is the transmission matrix for the rate of appearance of new infectious, V_t is the transition matrix for transfer rates among infected compartments, and $\rho(FV_t^{-1})$ represents the largest absolute eigenvalue of next-generation matrix FV_t^{-1}.

The SIMCI$_N$-based epidemiological parameter estimates can then be incorporated into the population dynamics describing grouper larvae exposed to NNV 30 days after 20-, 60- or 120-min bath immunization (i.e. the three vaccine groups), designated as the vaccination-based susceptible–infectious–mortality–carrier–natural/vaccinated immunity (SIMCI$_{NV}$) model (Equations T6–T10), to estimate the proportion of successful immunization (Table 1; Figure 1b).

per larvae, respectively. Therefore, in the SIMCI$_{NV}$ model, transmission rates can be decreased by ($1-p$) due to vaccination. Since the grouper larvae were exposed to NNV 30 days post-immunization, there were grouper populations that had acquired immunity in the beginning of the exposure, and the population in immunity state increased during the observation after exposure was likely due to natural immunity. To be consistent with this exposure scenario, we fitted the SIMCI$_{NV}$ model to the cumulative mortality data with initial condition {$S(0)$, $I(0)$, $M(0)$, $C(0)$, $I_{NV}(0) = 1-p, 0, 0, 0, p$}. Briefly, there are p proportion of population with vaccine-induced immunity in the I_{NV} state initially, the rest of population

(1−*p*) are susceptibles that are likely to be infected after NNV exposure, and the population in *I*, *M* and *C* states are zero in the beginning. Moreover, waning of immunity is not considered in the $SIMCI_{NV}$ model, since Kai and Chi (2008) had indicated that protection induced by the BEI-inactivated vaccine could maintain for three months after vaccination, enabling grouper larvae with the protection to grow into juveniles.

2.3 | Model calibration and sensitivity analysis

Normalized root-mean-square error (NRMSE) was adopted to assess performance of the transmission dynamic models and to calibrate simulation outcomes against the published data as,

$$\text{NRMSE}\,(\%) = \sqrt{\frac{\sum_{i=1}^{N_O}(P_i - O_i)^2}{N_O}} \times \frac{100\%}{\overline{O}},\text{ where } N_O \text{ denotes the number of}$$

observations, O_i is the experimental data, P_i is the modelled result, and $\overline{O}$ is the mean of experimental data. NRMSE values in the ranges of <10%, 10%–20% and 20%–30% indicate that the simulations are excellent, good and fair, respectively, whereas >30% is poor (Martins et al., 2018).

After obtaining epidemiological parameter estimates by fitting the transmission dynamic models to the cumulative mortality data, it is necessary to examine which epidemiological parameters in the transmission dynamic model have the most significant influence on cumulative mortality. Sensitivity analysis for epidemiological parameters (β_I, β_C, γ_I, *q*, γ_C) on cumulative mortality in the NNV–grouper larvae system can be performed at a specific time as, $\phi\,(t) = (M_2(t) - M_1(t))/\Delta$, where $\phi\,(t)$ is the sensitivity for one parameter at time *t*, $M_1(t)$ is the result of simulation of all parameters at specific values, $\Delta = 0.001 \times P_1$ presents the value of one parameter (P_1) that is adjusted by adding this amount, and $M_2(t)$ is the result of simulation with one adjusted parameter.

The sensitivity analysis was performed for each epidemiological parameter (β_I, β_C, γ_I, *q*, γ_C) in the control group and three vaccine groups. As a result, sensitivities for parameters could be compared within a single scenario or between different scenarios.

2.4 | Model for disease control

To provide a mechanistic strategy for farmer to prevent NNV outbreaks in grouper larvae by applying vaccine, fitted results of immersion time of bath immunization (t_b) and the proportion of successful immunization (*p*) were used to construct a vaccination-based model for disease control. The relationship between t_b and *p* estimates $p = f(t_b)$ can be obtained by a regression modelling technique. After constructing the t_b – *p* profile, the optimal t_b could be determined.

In addition, $p = f(t_b)$ can be incorporated into a vaccination-based model for disease control to quantify the effect of vaccination strategies on containing NNV transmission and can be written as Equation

T11, where R_C is the controlled reproduction number for NNV–grouper larvae system, p_b is the proportion of population to be bathed immunization (–), and *p* is the proportion of successful immunization as a function of specific immersion time of bath immunization (t_b, min) (–). R_0 for NNV transmission in grouper larvae without vaccination could be calculated based on Equation 1. Suitable strategies of bath immunization can be proposed by the vaccination-based model for disease control, resulting in $R_C < 1$ to control NNV transmission.

2.5 | Modelling scheme

Berkeley Madonna 8.0.1 (Berkeley Madonna was developed by Robert Macey and George Oster of the University of California at Berkeley) was applied to estimate epidemiological parameters by fitting the transmission dynamic models to the cumulative mortality data of grouper larvae. TableCurve 2D package (version 5.01, AISN software) was employed to perform the model fitting and obtain the optimal statistic models. The overall conceptual framework is demonstrated in Figure 2 depicting (a) problem formulation, (b) data collection/analysis and (c) the transmission dynamics modelling and control measure with vaccination strategies.

3 | RESULTS

3.1 | Parameter estimations

Result of the $SIMCI_N$ model fitting to the cumulative mortality data of grouper larvae exposed to NNV without vaccination showed a good agreement with a NRMSE of 6.81% (Figure 3a, Table S2). Results indicated that time for initial infectious population appearance occurred at day 4 after NNV exposure with a 0.01 proportion of initial infectious population in grouper larvae. The estimated transmission rates of infectious and carriers were 2.29 and 1.63 day^{-1}, respectively. The rate estimates for grouper larvae leaving infectious and carrier populations were 0.96 and 0.71 day^{-1}, respectively. The proportion of infectious population that became carriers was 0.02. In addition, R_0 estimate for grouper larvae exposed to NNV was 2.44, indicating that NNV would outbreak in a totally susceptible population.

On the other hand, the proportion of successful immunization (*p*) in grouper larvae with vaccination could be estimated after obtaining estimates of β_I, β_C, γ_I, *q* and γ_C (Table S2). Results of the vaccination-based $SIMCI_{NV}$ model fitting to the cumulative mortality data of grouper larvae exposed to NNV 30-day after 20-, 60- and 120-min bath immunization also showed a good agreement with NRMSEs of 8.30%, 15.81% and 6.23%, respectively (Figure 3b–d).

Results indicated that *p*s were 0.42, 0.94 and 0.52 in the immersion conditions of 20, 60 and 120 min, respectively (Table S3). Moreover, for immersion time of 60 min, estimates of the proportion of initial infectious population and the time for initial infectious population appearance were lower and later, respectively,

FIGURE 2 Conceptual framework for assessing NNV transmission dynamics in grouper larvae with vaccination control measure [Colour figure can be viewed at wileyonlinelibrary.com]
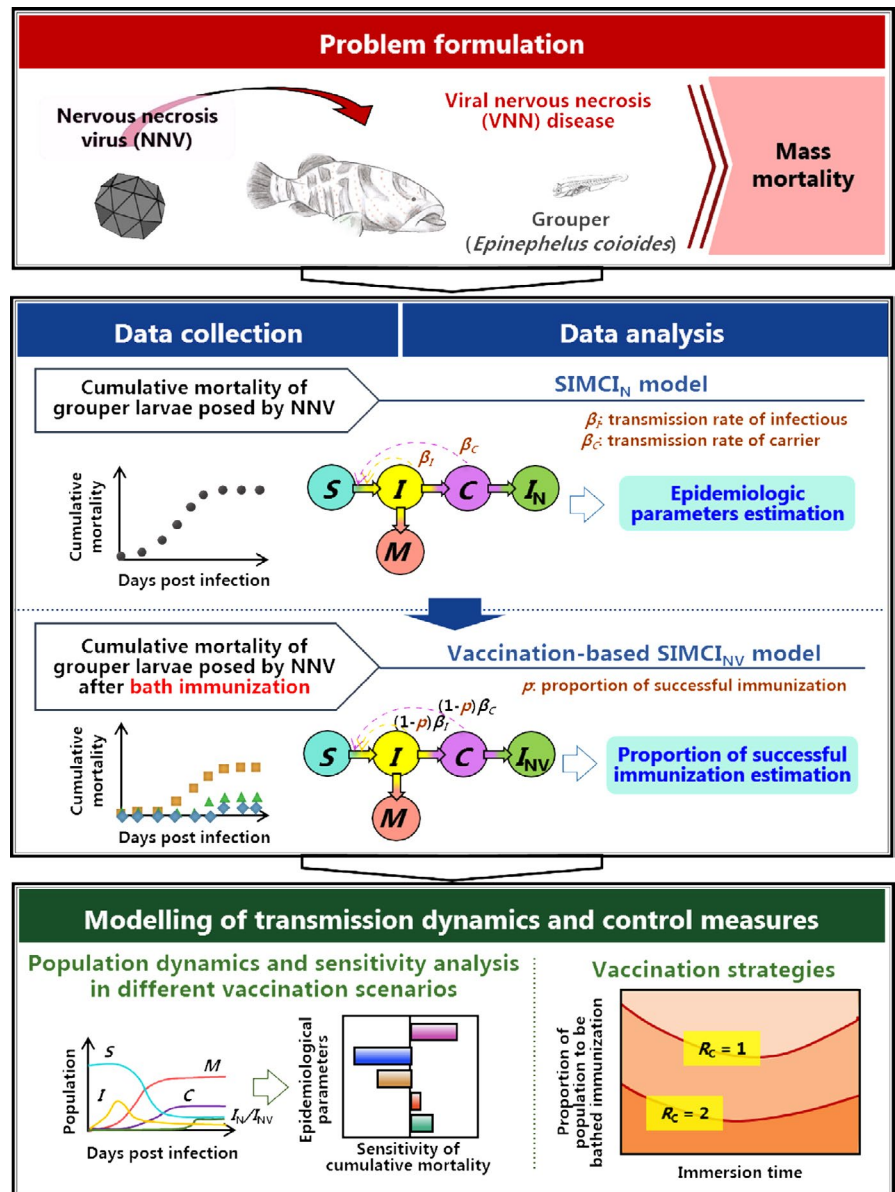
than those in the scenarios of 20- and 120-min bath immunization (Table S3).

3.2 | Population dynamics

Simulations of the population transmission dynamics indicated that proportion of susceptible population in grouper larvae without vaccination was decreased from 1 to 0.11 after 30 days (Figure 4a). On the other hand, for grouper larvae with 20-, 60- and 120-min bath immunization, proportions of susceptible population were 0.33, 0 and 0.35, respectively, at the day 30 (Figure 4a). The proportions of infectious population in grouper larvae without vaccination and with 20-, 60- and 120-min bath immunization reached the highest proportions of 0.22, 0.08, 0.03 and 0.04 at days 8, 4, 21 and 9, respectively (Figure 4b).

The proportion of mortality population in grouper larvae without vaccination was 0.86 at the day 30, whereas only 0.02 proportion of grouper larvae became carrier and were transferred into immune population (Figure 4c,d,e). Moreover, the immune population size of grouper larvae with vaccination of 20-, 60- and 120-min bath immunization remained nearly constants of 0.42, 0.94 and 0.52, respectively (Figure 4e). Results demonstrated that most of NNV-infected grouper larvae that were not successfully immunized were perished from disease instead of becoming carrier or immune population.

3.3 | Sensitivity analysis

We further tested for the sensitivity of our results to changes in core epidemiological parameter values of β_I, β_C, γ_I, q and γ_C (Figure 5).
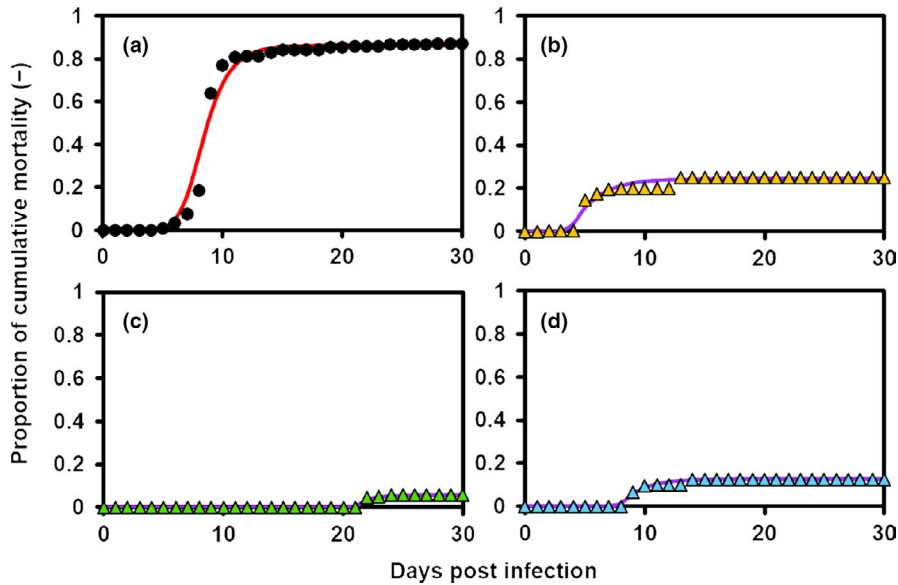
FIGURE 3 Fitted cumulative mortalities of grouper larvae exposed to NNV (a) without vaccination (the SIMCI$_N$ model) or 30 days after (b) 20-, (c) 60- or (d) 120-min bath immunization (the SIMCI$_{NV}$ model) [Colour figure can be viewed at wileyonlinelibrary.com]

Results showed that increments of q and γ_C were corresponding to negative changes of cumulative mortality, whereas increments of β_I and β_C were corresponding to positive changes of cumulative mortality (Figure 5). Specially, the effect of increasing γ_I on change of cumulative mortality in the scenario of 60-min bath immunization was contrary to those in the scenarios of without vaccination and 20- and 120-min bath immunizations. It was also found that cumulative mortality was more sensitive to β_I, γ_I and q, implying that these parameters were much more the fundamental drivers of the NNV transmission dynamics in grouper larvae (Figure 5). Moreover, sensitivities of cumulative mortality to most of parameters in the scenario of 60-min bath immunization were less significant than those in other scenarios (Figure 5).

3.4 | Vaccination to control disease

The relationships between immersion time (t_b) and proportion of successful immunization (p) in grouper larvae could be well fitted by a non-linear regression model following by a polynomial function as $p = f(t_b) = a + bt_b + ct_b^{2.5}$ where a is the intercept, and b and c are the fitted coefficients ($r^2 = 0.99$, $p < .001$) (Figure 6a; Table S4). Results showed that optimal immersion time for grouper larvae to generate immunity was 75 min (Figure 6a).

Furthermore, immersion time-associated vaccination strategies could be provided to control NNV transmission in terms of R_C (Figure 6b). Results indicated that there was a suitable range of immersion time (t_b) for a specific proportion of grouper larvae to be immunized (p_b) (Figure 6b). To effectively control NNV transmission by vaccination, there was at least 0.6 of proportion of population needed to be immunized for 70–80 min ($R_C = 1$) (Figure 6b). If there were 0.8 of proportion of population could be immunized, immersion times ranged from 40 to 110 min, whereas immersion times ranged from 30 to 115 min for all of the immunized grouper larvae (Figure 6b). Generally, the more proportion of population could be

immunized; the wider range of acceptable immersion time could be attained.

4 | DISCUSSION

Based on the results of population dynamics estimation, mortality of grouper larvae *E. coioides* without vaccination appeared 5 days post-NNV infection. Relative information regarding VNN disease transmission in the same species has been reported. Infected larvae were found to be died within 3 days, and the mortality levels could be even more higher than 99% (Chi, Lin, Su, & Hu, 1999; Chi, Shieh, & Lin, 2003; Kuo, Wang, Hsu, Chen, et al., 2012; Kuo, Wang, Hsu, Lee, et al., 2012; Wu, Chen, Lin, Tzeng, & Chang, 2012). In the laboratory-scale observations, cumulative mortality of grouper larvae at ambient temperature could reach 100% within 80 hr after NNV exposure (Chi et al., 1999).

However, the transmission pattern of VNN disease would be different in other species of grouper. For instance, the experimental infection trials of Malabar grouper ranging from 2 to 5 cm showed that the VNN disease was produced within 4–5 days and mortality ranged from 40% to 60% by 10 days post-infection under the rearing conditions of the hatchery (Boonyaratpalin, Supamattaya, Kasornchandra, & Hoffmann, 1996). Mortality levels of grouper *Epinephelus malabaricus* were also found higher in cage cultures along the shore with 50%–80% (Boonyaratpalin et al., 1996). For the redspotted grouper *Epinephelus akaara*, the earliest occurrence of diseases was 10–14 days after NNV exposure and fish died in another 3 days (Mori et al., 1991). Since the development of disease involves many factors including host, pathogen and environment, applying the SIMCI$_N$ model introduced here would be helpful for revealing the transmission dynamics of VNN disease in other species (Lavilla, 2001; Ögüt, 2001; Owens, 2012; Reno, 1988).
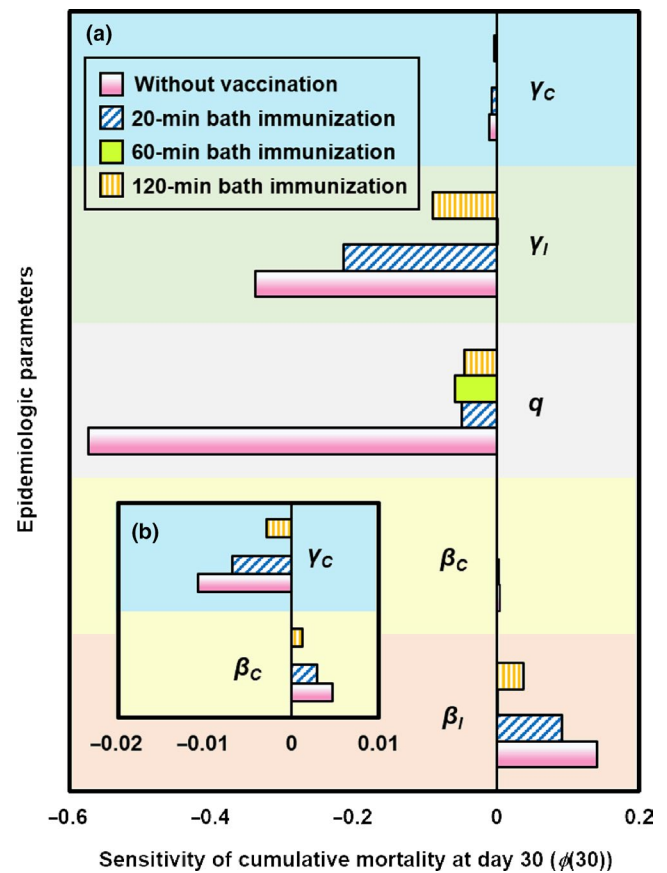
Mortality reduction is often considered as an indicator for vaccine programme effectiveness (Lahariya, 2016). Our modelling study
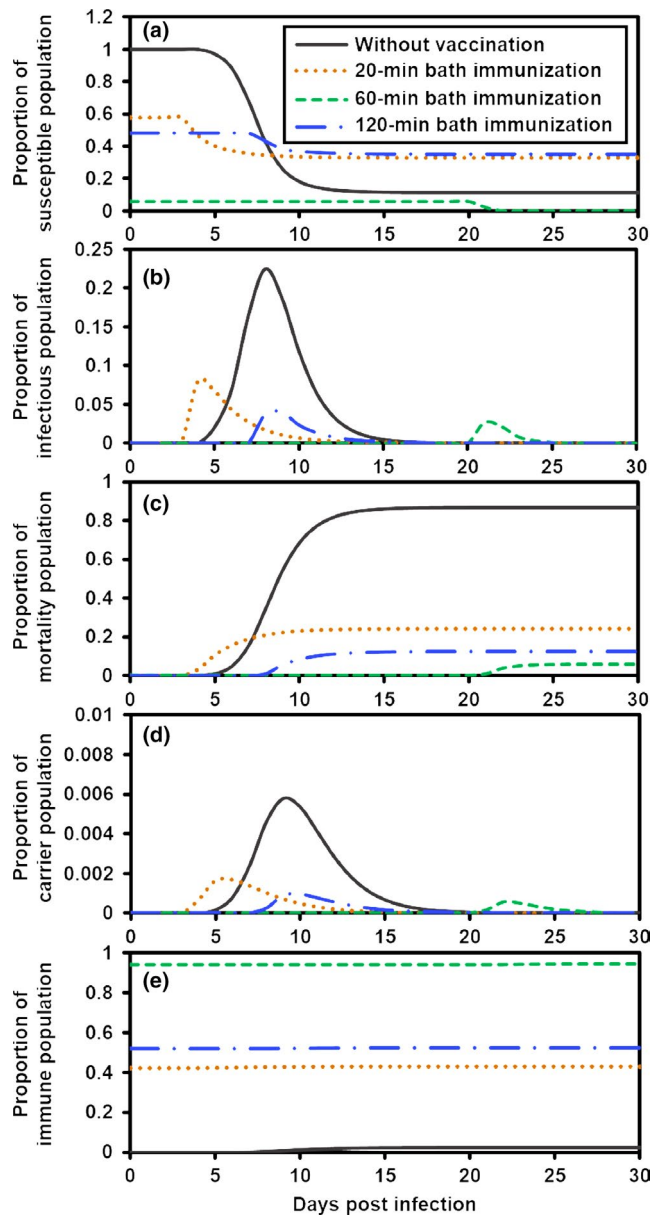
FIGURE 4 Simulation of the proportion of (a) susceptible, (b) infectious, (c) mortality, (d) carrier and (e) immune populations in grouper larvae exposed to NNV without vaccination or 30 days after 20-, 60- or 120-min bath immunization [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 5 (a, b) Sensitivity analysis Of cumulative mortality at day 30 (ϕ(30)) for grouper larvae exposed to NNV without vaccination or 30 days after 20-, 60- or 120-min bath immunization to transmission rate of infectious (β_I), transmission rate of carrier (β_C), proportion of infectious that become carriers (q), rate of individuals leave infectious population (γ_I) and rate of individuals leave carrier population (γ_C) [Colour figure can be viewed at wileyonlinelibrary.com]

predicted that cumulative mortality would decrease by more than half in grouper larvae exposed to NNV due to immersion in the vaccine solution. In an epidemiological concept, our results also showed the effect of herd immunity. The estimated proportion of population needed to be immunized to attain $R_C = 1$ actually represents the herd immunity threshold that is defined as the minimum proportion to be immunized in a population for elimination of infection (Lahariya, 2016).

Even though it has been reported that application of NNV vaccine could effectively reduce cumulative mortality of grouper larvae either in laboratory or in real farms, commercial NNV vaccine was still unavailable (Harikrishnan, Balasundaram, & Heo, 2011;

Hazreen-Nita, Azila, Mukai, Firdaua-Nawi, & Nur-Nazifah, 2019; Kai & Chi, 2008; Kai et al., 2014; Yamashita, Fujita, Kawakami, & Nakai, 2005). Before vaccine is commercialized, the key factors such as size, age and numbers of fish influencing vaccination should be optimized for designing immunization strategies (Hazreen-Nita et al., 2019; Sudheesh & Cain, 2017).

In this study, time for initial infectious population appearance was found to be influenced by immersion time. We showed that 60- and 120-min bath immunization would prolong the disease onset, whereas 20-min bath immunization would be ahead of the time for disease onset. Moreover, the proportion of successful immunization induced by 60-min bath immunization was higher than those in the scenarios of 20- and 120-min bath immunization, implicating that there was a suitable range of immersion time for grouper larvae for acquiring an effective protection. From the economic point of view, control strategies that require the lowest costs with highest impact are desirable (Sitjà-Bobadilla & Oidtmann, 2017). Our finding could help farmer to decide how many grouper larvae would be vaccinated based on their available capital and resources
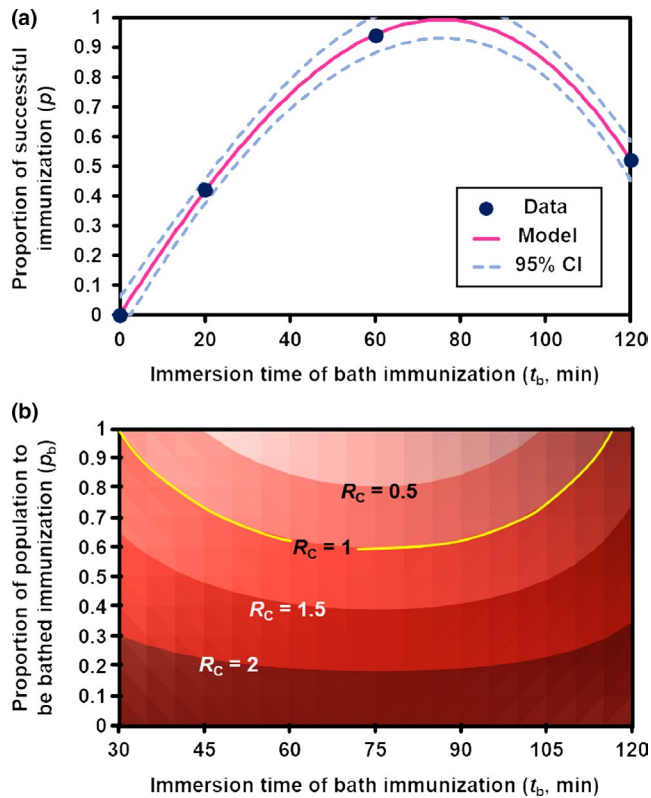
FIGURE 6 (a) The relationship between immersion time of bath immunization and the proportion of successful immunization. (b) Contour plot of R_C in the strategies of bathing immunization for different proportions of population with different immersion times [Colour figure can be viewed at wileyonlinelibrary.com]

and to know how long the grouper larvae should be immunized based on the proportion of population to be bathed immunization that they adopted.

Length of immersion time was identified as a key factor determining the immune efficacy (Du, Tang, Sheng, Xing, & Zhan, 2015). Kai et al. (2014) indicated that bath vaccinations could trigger the Mx gene expression of immunity in grouper larvae. The Mx gene expression was found to be triggered by type I interferon (IFN), a type of cytokines, which was expressed as the first line of defence against viral infections and played the role in limiting disease spread during the onset of infection (Chen, Kuo, et al., 2014; Chen, Wang & Chen, 2014). NNV-infected fish were most likely to limit virus replication via increasing the production of IFN and IFN-stimulated genes to initiate the innate immune response (Chen, Wang, & Chen, 2014; Lu & Wu, 2007). Therefore, 60-min bath immunization is highly likely to induce higher expression of immune gene than 20- and 120-min bath immunization to limit the spread of VNN disease in grouper larvae.

Other factors, such as temperature, environmental stressors, stocking density and fish handling, which might cause immune suppression during vaccination were not considered in this study (Hazreen-Nita et al., 2019; Muktar, Tesfaye, & Tesfaye, 2016; Snieszko, 1974). For stocking density, grouper larvae used in this study were cultured at the density of 2 fish L^{-1}, which was extremely

lower than that ranging from 20 to 50 fish L^{-1} in real farms but was close to that was suggested under threat of viral infection (Pierre et al., 2000; Rimmer, 2000), implying that this relative low density might be adequate for grouper larvae during vaccination. The effects of these factors on the vaccine effectiveness can be comprehensively investigated in the future to establish the appropriate and adjustable vaccination strategies before applying NNV vaccine for grouper larvae in farming practices.

Nevertheless, based on the results of sensitivity analysis, the key epidemiological parameters that affect the population dynamics of grouper larvae were identified to provide an additional insight of controlling NNV transmission via vaccination. It is worthy to be mentioned that the changes of most of parameters for grouper larvae vaccinated via 60-min bath immunization had less effects on cumulative mortality, implying that effective protection could reduce the impact of epidemiological parameters on disease transmission.

The usual onset of disease and the latest occurrence of VNN on larval and juvenile grouper were documented as the total body length of 9–10 mm and <40 mm, respectively (Munday, Kwang, & Moody, 2002). In other words, groupers between these life stages were vulnerable to VNN disease. After vaccination, the protection induced by the BEI-inactivated vaccine enabled grouper larvae growth into juveniles with total body length of 10–12 cm, whereas in juvenile stage, fish should be re-immunized by other vaccines via injection (Kai & Chi, 2008).

However, although R_C for NNV transmission in grouper larvae could decrease to less than 1 by immersing with vaccines, it needed sufficient time for grouper larvae to develop efficient immunity. Kai and Chi (2008) reported that 15 days were not enough for larvae to develop immunity. Furthermore, it was not suitable for vaccinating grouper larvae when they were premature since the immune system of fish was not well developed (Harikrishnan et al., 2011; Shetty, Maiti, Santhosh, Yenugopal, & Karunasagar, 2012; Sudheesh & Cain, 2017). Hence, proper husbandry and biosecurity along with effective control strategies for grouper larvae before and after vaccination are essential to be adapted in real farms.

In the duration that grouper larvae are unable to be vaccinated or have not developed effective immunity, farmers can reduce the NNV infection risk for larvae by preventing horizontal and vertical transmissions. Horizontal transmission is most likely to occur via infected fish, contaminated feed and bird faeces, and contaminated water (Chi et al., 2016; Kuo, Wang, Hsu, Lee, et al., 2012; Skliris & Richards, 1998). As a result, the potential strategies such as disinfection of transport vehicle, rearing water or live feed and maintaining quarantine of ill fish are necessary (Chi et al., 2016; Shetty, Maiti, Santhosh, Venugopal, & Karunasagar, 2012). On the other hand, to effectively reduce lateral transmission, maintenance of the larvae at less than 10 L^{-1} in ponds has shown commercially viable (Munday & Nakai, 1997).

For vertical transmission, NNV was found to spread from the infected broodfish to the offspring (Breui, Pépin, Boscher, & Thiéry, 2002; Mushiake, Nishizawa, Nakai, Furusawa, & Muroga, 1994). It

was also revealed that NNV existed in embryos and the amount of virus was likely to increase during the development even after hatching (Kuo, Wang, Hsu, Chen, et al., 2012). To reduce the risk of vertical transmission, grouper broodfish should be vaccinated and the eggs could be washed by ozonated water to inactivate NNV attaching on the surface (Huang et al., 2017; Shetty et al., 2012).

Technically, a convenient real-time PCR method was successfully applied rapidly and sensitively for detecting NNV infection and latent NNV in normal grouper with total body length of 10–30 mm (Yinnan, Kebing, Xinhua, & Jingqun, 2013). Furthermore, an automated microfluidic chip system could be used to identify the presence of NNV with plausible virus vectors around fish farms (Kuo, Wang, Hsu, Lee, et al., 2012). Epidemic alerts provided by these methods are capable of allowing farmers to take early intervention measures to reduce the economic losses (Yinnan et al., 2013). When application of these techniques is as one of the strategies in integrated pathogen management (IPM) or integrated coastal zone management (ICZM) (Chen & Qiu, 2014; Sitjà-Bobadilla & Oidtmann, 2017), the performance obtained after the implementation of various strategies will aid in the modelling of disease and then improve the decision-making processes (Sitjà-Bobadilla & Oidtmann, 2017). To achieve this purpose, parameters which are able to quantify the performance of the implementation could be incorporated into a transmission dynamic model.

Notably, although the potential model for disease control proposed in this study could be used to assess the effectiveness of vaccination strategies, control measure specifically focused on BEI-inactivated vaccine that provided for grouper larvae immunization. Effectiveness of application of other developed NNV vaccines should also be assessed in the future. In addition to vaccination, immunostimulants such as probiotics also provide a practical measure in controlling diseases (Harikrishnan et al., 2011; Magnadottir, 2010). Future study could dedicate to investigate the key elements in these processes such as immunoglobulin M activation that plays an important role in against viral infection to reduce mortality in grouper larvae. Our findings also set the stage for future studies exploring aquaculture management strategies on VNN infection risk, particularly in farms most likely to pose VNN disease threat in the future, and put the focus on identifying potential economic losses and early warning indicators for them.

Along with recent studies dealing with VNN disease in grouper, our study provides a well-defined framework for quantifying the NNV transmission dynamics with vaccination strategies that can inspire more novel mechanistic models to explore fish responses to new emerging diseases. We conclude that our data-driven modelling approach that links the transmission dynamics of NNV and vaccination strategies for grouper has the potential to support evidence-based planning and adaptation of integrated control measures. We encourage that the epidemiology-based framework introduced here can be used to identify those attributes for which the responses to NNV transmission are more sensitive to buffering and for establishing effective vaccination and mitigation actions aimed at controlling VNN disease in fish farming practices.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Datasets of proportion of cumulative mortality for grouper larvae exposed to NNV via immersion without vaccination or 30-day after 20-, 60- or 120-min bath immunization were extracted from Kai and Chi (2008).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.