Aquaculture 529 (2020) 735670

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Novel insights into the selective breeding for disease resistance to vibriosis by using natural outbreak survival data in Chinese tongue sole (*Cynoglossus semilaevis*)

Yuanri Hu[a], Yangzhen Li[a,*], Zhongming Li[b], Changshan Chen[c], Jiajian Zang[c], Yuwei Li[c], Xiangqing Kong[d]

[a] Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Laboratory for Marine Fisheries Science and Food Production Processes, Pilot Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China
[b] Weifang Sanxin Aquatic Technology Development Co., Ltd., Weifang 261311, China
[c] Rizhao Marine Aquatic Resources & Propagation Co., Ltd., Rizhao 276805, China
[d] Fisheries Technology Promotion Station of Donggang District, Rizhao 276862, China

ARTICLE INFO

Keywords:
Tongue sole
Cynoglossus semilaevis
Genetic parameter
Heritability
Vibriosis
Disease resistance

ABSTRACT

As an important farmed flatfish species, Chinese tongue sole (*Cynoglossus semilaevis*) is heavily threatened by vibriosis in recent years. Genetic selection for disease resistance is a sustainable and effective approach to reduce frequent outbreaks. To verify whether the resistance of vibriosis (caused by co-infections of different *Vibrio* spp. in natural outbreak) can be included in breeding programs, in this study, genetic analysis of resistance to vibriosis based on natural outbreak survival data was carried out by using four statistical models (three cross-sectional models and one longitudinal model). The magnitude of the genetic variation in the resistance of vibriosis was estimated through a 56-day natural outbreak test of 15,912 individuals from 78 full-sib families (the offspring of 77 sires and 78 dams). Variance components and heritabilities were estimated at two cut-off points respectively, i.e. day 35 with 49.2% cumulative mortality and day 56 with 71.6% finial cumulative mortality. Heritabilities of resistance to vibriosis were low to moderate, where values at day 56 (0.04–0.21) were significantly different from zero, while values at day 35 (0.03–0.10) were not significantly different from zero in each corresponding model. The Spearman rank correlations between family EBVs for different models were high (> 0.98), indicating a nearly identical ranking of families. Compare to three simpler cross-section models, the longitudinal model taken the time until death into account demonstrated the highest accuracy of family selection. These results confirmed the existence of genetic variation for resistance to vibriosis and provided novel insights into the selective breeding for disease resistance to vibriosis by using natural outbreak survival data in tongue sole.

1. Introduction

Chinese tongue sole (*Cynoglossus semilaevis*) is an indigenous marine flatfish species which is widely distributed in China's north coastal areas (Guan et al., 2018). Tongue sole has been overfished since the 1990s, and indoor farming began in 2003 then with a rapid development of aquaculture industry (Guan et al., 2018). Now, tongue sole contributes to a large part of flatfish species production and is one of the most expensive farmed fish species on the market in China (Song et al., 2020; Guan et al., 2018). With the development of production scale and intension of aquaculture industry of tongue sole, high-stress rearing environmental conditions such as high stocking density, inadequate nutrition and problems related to water quality should be responsible for frequent disease outbreaks with significant production losses.

Vibrio spp. as important global fish pathogenic bacteria with a wide range of aquatic hosts (Mohamad et al., 2019), have emerged as the most important disease in tongue sole in China, an epidemic with a high mortality rate (50%–70%) recently observed mainly in juvenile fish (body length between 10 cm and 20 cm) at relatively high summer temperatures (Li et al., 2019; Zhang et al., 2015; Tang et al., 2008). Symptoms of vibriosis of tongue sole included surface ulcers, ascites, tail rot, eye infection and septicemia. Indeed, *Vibrio* spp., such as *V. anguillarum*, *V. harveyi*, *V. vulnificus*, *V. salmonicida*, *V. alginolyticus*, *V. parahaemolyticus*, *V. rotiferianus*, *V. ponticus* that are known to cause

* Corresponding author.
E-mail address: liyz@ysfri.ac.cn (Y. Li).

https://doi.org/10.1016/j.aquaculture.2020.735670
Received 4 May 2020; Received in revised form 28 June 2020
Available online 08 July 2020

devastating impacts on cultured marine fishes worldwide due to their strong pathogenicity and ubiquitous presence in marine environment (Mohamad et al., 2019). Some *Vibrio* species are opportunistic pathogens to cultured fishes and can co-infect within the same host (Liu et al., 2016a; Kim et al., 2014; Gauger et al., 2006).

The common methods to control, prevent and treat vibriosis in aquaculture are by using disinfectors, vaccines and antibiotics. However, the *Vibrio* spp. pathogens have proven difficult to eradicate and prolonged misusage use of antibiotics in farms has resulted in limited success and antibiotic resistant strains (Nguyen et al., 2017; Rao and Lalitha, 2015; Song et al., 2014; Cabello et al., 2013; Magnadottir, 2010). In aquaculture, for obtaining a continuous and permanent genetic gain, the selective breeding has been proved a more effective approach to enhance disease resistance (Ødegård et al., 2011; Yáñez et al., 2014). Selective breeding for resistance to vibriosis have been well studied in several aquaculture species such as Atlantic cod (*Gadus morhua* L.) (Bangera et al., 2011, 2013; Kettunen et al., 2007; Kettunen and Fjalestad, 2006), clam (*Meretrix petechialis*) (Liang et al., 2017), turbot (*Scophthalmus maximus*) (Wang and Ma, 2019), Pacific oyster (*Crassostrea gigas*) (Azéma et al., 2017).

Currently, for most aquaculture species, disease resistance is generally assessed based on challenge test data (Ødegård et al., 2011). Evidence showed that challenge test and natural infection may share a similar molecular mechanism which could be inferred from the high genetic correlation between experimental challenge and field outbreak data (Gjøen et al., 1997; Wetten et al., 2007). Hence, it is expected that the improvement of disease resistance either by challenge test or natural infection will be transferred to the commercial populations. For genetic analysis of survival traits in aquaculture species, standard statistical approaches and methodologies have been developed, including survival trait definitions and corresponding statistical models, for example case studies by Bangera et al. (2014), Yáñez et al. (2013), Ødegård et al. (2010a, 2011), Ødegård et al. (2006), Gitterle et al. (2006).

Selective breeding programs for disease resistance have been already established in tongue sole, mainly targeted at vibriosis (*V. harveyi* and *V. anguillarum*) (Li et al., 2019; Liu et al., 2016b) and edwardsiellosis (*Edwardsiella tarda*) (Li et al., 2020b; Liu et al., 2016b) based on challenge test data. However, genetic parameters and selection response about natural outbreak survival are still not available. To address this knowledge gap and include natural outbreak disease resistance into the breeding objective, the estimation of heritability is needed to understand whether genetic variation occurs for this desirable trait. So, in this study we aimed at estimating genetic parameters for resistance to vibriosis by using valuable natural outbreak survival data.

2. Materials and methods

2.1. Fish material

Methods of family production and maintenance were as described by Li et al. (2019). Finally, 78 families (78 dams and 77 sires) were used in this study. In the early month of August 2014, approximately 200 individuals (total length 8–10 cm) from each family were random sampled, anesthetized and tagged (by family) with a visible implant elastomer (VIE) (Qingdao Starfish Instruments Co., Ltd). Before tagging, each family was reared in a separate tank. Tagging work completed on 8th August. All the tagged fish (n = 16,000) were randomly divided into two equal batches (i.e., about 100 fish per family per batch) and transferred to four concrete flow-through rearing tanks (6 × 6 × 1.2-m, L × W × H) (each batch in two tanks) under commercial aquaculture conditions for later monitoring disease outbreaks. Fish were fed with commercial pellet feed twice daily. Water temperatures were maintained at 21–25 °C. Salinity was 28–30‰, water exchange rate was 400% per day and dissolved oxygen was 6–8 mg/L.

Table 1
Descriptive statistics of the dataset used.

Item	
Full-sib families, no.	78
Sires, no.	77
Dams, no.	78
Fish no. with data	15,912
Batches	2
Average family sample size of each batch	103
Final mortality (%)	71.6
Range of family mortality (%)	41.7–92.6
Outbreak period (days)	56

On 30th August 2014, mortalities were observed due to an infection, which was verified as vibriosis infection by isolating bacteria from the skin ulceration and kidneys of the randomly selected mortalities with typical symptoms. All the dead individuals showed clear clinical signs. The identification was performed by means of biochemical characterization and multiplex PCR-based protocols which were similar to Cano-Gomez et al. (2015). The main pathogenic bacteria were identified as *V. harveyi* (42%), *V. vulnificus* (20%), *V. anguillarum* (15%), *V. parahaemolyticus* (12%) and other unknown species (11%). Dead and moribund fish were removed and recorded twice daily until 24th October 2014. During disease outbreak period, no drug (antibiotics or other herbal extracts) was used. The natural mortality of fish prior to disease outbreak period was excluded. More detailed information was showed in Table 1.

2.2. Trait definition

Resistance to vibriosis was recorded as two different trait definitions:

1. Binary test survival (BTS), which was scored 0 if the fish died prior to cut-off day and scored 1 elsewhere. In this study, we set test day 35 and day 56 as cut-off point days, when the cumulative mortalities were 49.2% and 71.6% respectively.
2. Binary test day survival (BTDS), with one record per day the fish stayed in the test and the number of records per fish equals the number of days until death. The observation was scored 0 if the fish died on the actual test day and scored 1 if the fish was alive on the actual test day until death or censoring. The test day of first mortality was set as a starting point (Ødegård et al., 2007; Gitterle et al., 2006).

2.3. Survival analysis

The Kaplan-Meier survival function (Kaplan and Meier, 1958) was implemented to plot survival curves for all families, the best family, the worst family and all families of each batch respectively by using R software (version 3.6.1). The survival distribution function $\widehat{S}(t)$ is expressed as follows:

$$\widehat{S}(t) = \prod_{t_i \le t} \left(1 + \frac{d_i}{n_i}\right)$$

where t_i is time at point i, d_i is the number of fish that had died at t_i, and n_i is the number of fish surviving (at risk) just prior to t_i.

At the end of the test, observations (survival) were recorded as censored. They were assumed to die sometime after the end of the test. The nonparametric log-rank, Breslow and Tarone-Ware tests were used to test equality of the survival functions. Then a Cox proportional hazards regression analysis (Cox, 1972) was used to measure the survival time of the families. The Cox proportional hazard function is: $h(t, X_i) = h_0(t) \times \exp(\alpha' t_j + \beta' f_k)$ where $h(t, X_i)$ is the hazard function of fish at a certain time, $h_0(t)$ is the baseline hazard function when all risk

factors are zero, α' and β' are the regression coefficients, t_j is the fixed effect of tank, f_k is the fixed effect of family. Families were grouped to resistant and susceptible based on hazard ratio ($HR = \frac{h_i(t)}{h_r(t)}$, where $h_i(t)$ is the mortality risk in family i and $h_r(t)$ is the mortality risk in the reference family with 0% survival) using SPSS software (version 23.0). Families with hazard ratio < 1 were classified as resistant, and otherwise they were susceptible.

2.4. Statistical model

The variance components and estimated breeding values (EBVs) were analyzed with four sire-dam models based on restricted maximum likelihood (REML) algorithm by using ASReml-R4 package (Butler et al., 2017). The models are as follows:

(1) Cross-section liner model (CLM) for BTS:

$$y_{ijkl} = \mu + f_j + s_j + d_k + c_{jk} + e_{ijkl}$$

where y_{ijkl} is the phenotypic observation for the trait; μ is the overall mean; f_j is the fixed effect (batch and tank); where s_j is the random genetic effect of sire j; d_k is the random genetic effect of dam k; c_{jk} is the effect of common full-sib family jk (including common environmental effect due to the separate rearing of the families before tagging, maternal effects and the non-additive genetic effects common to full-sibs) (Ødegård et al., 2007); e_{ijkl} is residual effect.

(2) Cross-sectional threshold (probit) model (CTMp) for BTS:

$$\Pr(y_{ijkl} = 1) = \Phi(\mu + f_j + s_j + d_k + c_{jk})$$

where $\Phi(.)$ represents cumulative standard normal distribution; other parameters are as described above. The underlying residual variance was 1.0.

(3) Cross-sectional threshold (logit) model (CTMl) for BTS:

$$Pr(y_{ijkl} = 1) = \frac{\exp(\mu + f_j + s_j + d_k + c_{jk})}{1 + \exp(\mu + f_j + s_j + d_k + c_{jk})}$$

where all the parameters are as described above. The residual variance was assumed to be $\pi^2/3$.

(4) Linear (logit) repeatability model (LRM) for BTDS:

$$Pr(y_{ijkl} = 1) = \frac{\exp\left(\sum_{p=0}^{1} b_p Z_p(t) + f_j + s_j + d_k + c_{jk}\right)}{1 + \exp\left(\sum_{p=0}^{1} b_p Z_p(t) + f_j + s_j + d_k + c_{jk}\right)}$$

where $Z_p(t)$ is a p^{th} order orthogonal polynomial of t (time at recording in days); b_p is the p^{th} order fixed regression coefficient; and the other parameters are as described above.

Full pedigree information was included in all genetic analyses. For all the models, the individual effects were equal to the total additive genetic effects, and were assumed to be $\sim N(0, A\sigma_a^2)$; the additive genetic sire and dam effects were assumed $\sim N(0, A\sigma_{sd}^2)$, $\sigma_{sd}^2 = \sigma_s^2 = \sigma_d^2 = 1/4\sigma_a^2$, which can be obtained in ASReml-R4 package though the model function 'and (vm (Dam, ainv))'; common full-sib family effects were assumed $\sim N(0, I\sigma_c^2)$, and residuals were assumed $\sim N(0, I\sigma_e^2)$; where A is the additive genetic relationship matrix, I is the identity matrix, σ_a^2, σ_s^2, σ_d^2, σ_{sd}^2, σ_c^2 and σ_e^2 is additive genetic variance, additive genetic sire variance, additive genetic dam variance, additive genetic sire-dam variance, common full-sibs variance and residual variance (assumed to be $\pi^2/3$ for CTMl and LRM) respectively. For all the models, heritability (h^2) was calculated as $h^2 = 4\sigma_{sd}^2/(2\sigma_{sd}^2 + \sigma_c^2 + \sigma_e^2)$.

2.5. Model comparison

For challenge test and natural outbreak survival data in aquaculture species, criteria and methods of model comparison have been clearly expounded by previous studies (Bangera et al., 2014; Yáñez et al., 2013; Gitterle et al., 2006; Ødegård et al., 2006, 2007, 2010b). In this study, briefly, the mid-parent full-sib family EBV's were independently predicted from the two replicated batches (subsets) using all the models at day 35 and day 56 respectively, and the accuracy (r_τ) of full-sib family EBVs are approximately equal to the Pearson correlation coefficients between the mid-parent full-sib family EBVs (r_{EBV}) predicted from two subsets. Given that predicted breeding values for the two subsets are independent and the prediction of breeding values is unbiased, the selection index can be shown as: $r_{EBV} \approx r_\tau^2$ (Gitterle et al., 2006). In addition, Spearman (rank) correlations between the full-sib family EBVs for each model were calculated to assess the agreement between genetic predictions of the different models (Gitterle et al., 2006).

2.6. Genetic correlation between natural survival and challenge test survival

In our previous study, *V. harveyi* challenge test survival data of 50 families (from 78 families in this study) have been obtained (Li et al., 2019). Hence, the genetic correlation (r_{xy}) between vibriosis natural outbreak survival and *V. harveyi* challenge test survival (treated as two different traits) was evaluated based on the datasets from common 50 families by using CLM based on bivariate analysis. The genetic correlation was calculated as follows (Falconer and Mackay, 1996):

$$r_{xy} = \frac{\sigma_{ax,ay}}{\sqrt{\sigma_{ax}^2 \sigma_{ay}^2}}$$

where $\sigma_{ax,\ ay}$ is the genetic covariance between the two tests, σ_{ax}^2 and σ_{ay}^2 were the genetic variance of the two survival traits respectively.

3. Results

3.1. Descriptive statistics of survival

Cumulative mortality, mean family survival and minimum and maximum of family survival in two batches at day 35 and day 56 for natural vibriosis outbreak are given in Table 2. Kaplan-Meier survival curves of the best and the worst family and all the families and two replicated batches during the outbreak period were shown in Fig. 1. At day 35 and 56, the cumulative mortalities across all the families were 49.2% and 71.6% respectively. Survival rates varied considerably among families, which indicated a substantial genetic variation related to resistance to vibriosis. It is worth mentioning that the mortality patterns were significantly different for the two batches (Log-Rank testing, $P < .0001$; Breslow testing, $P < .0001$). According to the

Table 2
Cumulative mortality (%), mean family survival (%) and minimum and maximum of family survival (%) in two batches at day 35 and day 56 for natural vibriosis outbreak in *C. semilaevis*.

Day	Cumulative mortality (%)	Batch	Mean family survival (%)	Min (%)	Max (%)
35	49.2	1	51.9	30.4	76.5
		2	49.6	24.5	77.5
56	71.6	1	30.5	4.9	60.8
		2	26.2	0.0	58.8

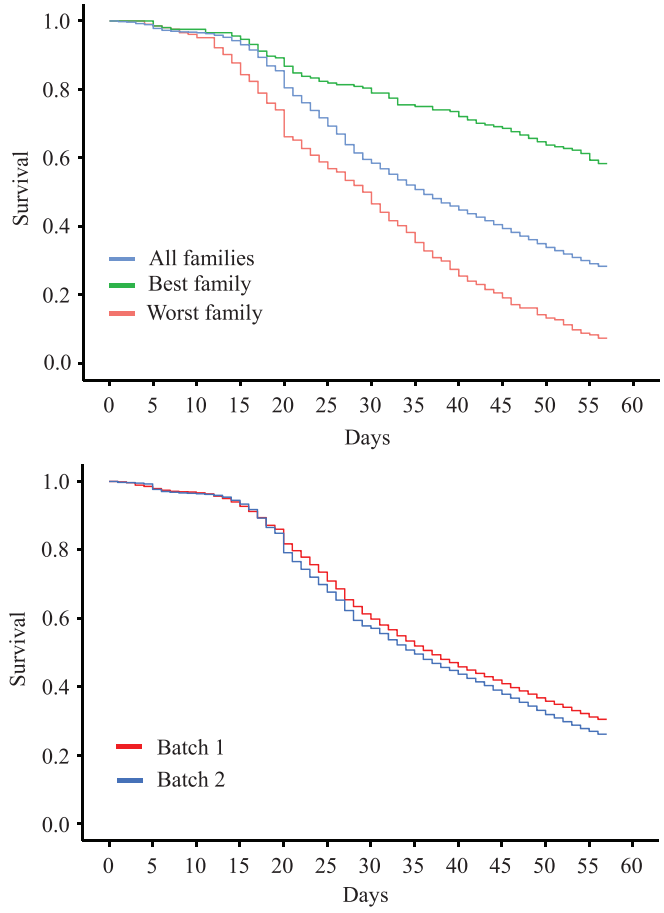

Fig. 1. Kaplan-Meier survival curves of the best and the worst family and all the families (above) and two replicated batches (below) during 56-day natural outbreaks of vibriosis in tongue sole.

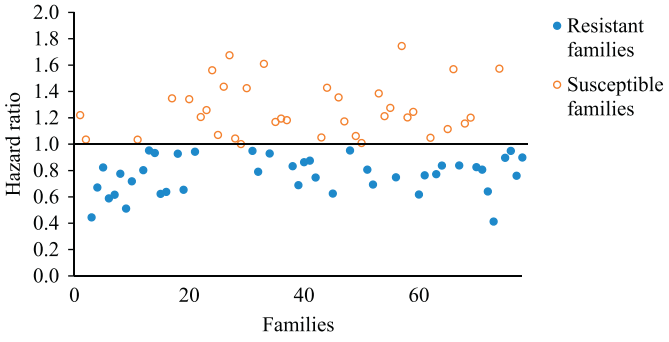

Fig. 2. Hazard ratio plot based on Cox regression analysis for vibriosis natural outbreak survival. Families with hazard ratio < 1 were classified as resistant, while families with hazard ratio > 1 were classified as susceptible.

results of Cox proportional hazards regression (Fig. 2), 42 families were classified as being in the resistant group (overall survival rate was 37.1%), while 36 families were classified as being in the susceptible group (overall survival rate was 18.2%).

3.2. Genetic parameters

Variance components and estimates of heritability (h^2) for different models are presented in Table 3. Heritability estimates were low to moderate (ranging from 0.03 to 0.21) and were highly variable and non-comparable. This was as expected and should be ascribed to different definitions, different cut-off points (days) and modeling.

Table 3
Estimated additive genetic sire-dam variance (σ_{sd}^2), common to full-sib family variance (σ_c^2), residual variance (σ_e^2) and heritability (h^2) with standard error (± SE) for vibriosis natural outbreak survival at day 35 and 56 (cumulative mortality was 49.2% and 71.6% respectively) in four models.

Model	Day	σ_{sd}^2	σ_c^2	σ_e^2	h^2 ± SE
CLM	35	0.004	0.0018	0.24	0.06 ± 0.05
	56	0.006	0	0.19	0.12 ± 0.02
CTMp	35	0.025	0.014	1	0.10 ± 0.08
	56	0.17	0	1	0.21 ± 0.03
CTMl	35	0.065	0.036	3.29	0.08 ± 0.07
	56	0.17	0	3.29	0.18 ± 0.03
LRM	35	0.027	0.013	3.29	0.03 ± 0.03
	56	0.032	0	3.29	0.04 ± 0.01

Heritabilities estimated from LRM were considerably lower than from other models at corresponding days. At day 56, for all the models, the heritability values were significantly different from zero ($P < .05$) and substantially larger than values at day 35 which were not significantly different from zero ($P > .05$). This is explained by the fact that when overall mortality increased from 49.2% to 71.6%, family mortality variance increased from 0.0109 to 0.0134. Genetic correlation between vibriosis natural outbreak survival and *V. harveyi* challenge test survival was 0.06 ± 0.05, not significantly different from zero (P > .05).

3.3. Model comparison

The estimates of Spearman correlation coefficients between full-sib family EBVs for the different models based on the full dataset are showed in Table 4. The correlations between LRM and other three models (0.98) were slightly lower than the correlations among three cross-sectional models (i.e. CLM, CTMp and CTMl) (~1). Very high correlations indicating nearly identical ranking of families for all the four models. Table 5 shows the estimates Pearson correlation coefficients between predicted full-sib family EBVs (r_{EBV}) from two batches of survival data and accuracy of selection (r_t) in four different statistical models at day 35 and 56. At both cut-off days, the correlations and accuracies of selection were very similar within the same models. Longitudinal model (LRM) performed slightly better than other three cross-sectional models in terms of predictive ability at each corresponding cut-off day.

4. Discussion

In aquaculture, frequent disease outbreaks have caused tremendous economic losses, reportedly by up to tens of billion dollars in the last two decades (FAO, 2015; FAO, 2016). Therefore, disease resistance as an economically important trait should be included in the breeding goal. Conventionally, for aquaculture fishes, selective breeding for improved disease resistance is mainly based on challenge test by injection with specific pathogens isolated from diseased fish with typical symptoms in controlled environments (Ødegård et al., 2011). Substantial genetic responses of disease resistance by challenge test based

Table 4
Spearman rank correlation coefficients[a] between full-sib family EBVs analyzed with four statistical models for vibriosis natural outbreak survival with the full dataset.

Model	CTMp	CTMl	LRM
CLM	0.9994	0.9993	0.9817
CTMp		0.9999	0.9826
CTMl			0.9818

[a] All the correlation coefficients were significantly different from zero. ($P < .001$).

Table 5
Estimated Pearson correlation coefficients[a] between predicted full-sib family EBVs (r_{EBV}) from two batches of survival data and accuracy of selection ($r_\tau = \sqrt{r_{EBV}}$) in four different statistical models at day 35 and 56 (cumulative mortality was 49.4% and 71.6% respectively) in four models.

Model	Day	r_{EBV}	r_τ
CLM	35	0.570	0.755
	56	0.587	0.766
CTMp	35	0.571	0.756
	56	0.583	0.764
CTMl	35	0.571	0.756
	56	0.583	0.764
LRM	35	0.593	0.770
	56	0.599	0.774

[a] All the correlation coefficients were significantly different from zero. ($P < .001$).

on intraperitoneal (IP) injection have been achieved in many species, such as Atlantic salmon (Yáñez et al., 2013; Ødegård et al., 2007), Atlantic cod (Ødegård et al., 2010b), rainbow trout (Leeds et al., 2010), red tilapia (Sukhavachana et al., 2019), whiteleg shrimp (Huang et al., 2012). However, this approach bypasses the first defense mechanisms (mucus and skin) of the animal, so this artificial type of challenge is not as 'realistic' as the natural. Further studies are necessary to demonstrate if resistance and infection mechanisms involved in challenge tests by injection are similar to natural infection. An alternative approach for challenge test is by cohabitation or bath challenge that the immune response may similar to natural infection. For vibriosis selective breeding in aquaculture fishes, survival data is mainly obtained based on IP injection and bath challenge methods, for example case studies in Atlantic cod (Kettunen et al., 2007; Kettunen and Fjalestad, 2006; Bangera et al., 2011, 2013). However, the selection response of vibriosis resistance based on natural field outbreak survival data remain poorly understood.

As demonstrated by the results of our work, the natural outbreak of vibriosis posed a severe threat to juvenile tongue sole, with a 71.6% cumulative mortality and a long breakout period (56 days). Considerable phenotypic variations of survival have been detected, i.e., cumulative family mortalities ranging from 41.7% to 92.6%, which suggests that resistance to vibriosis caused by different *Vibrio* spp. can be genetically improved by selective breeding. Based on natural outbreak survival data, significant genetic variation for resistance to vibriosis was detected and the magnitude of estimated heritabilities were low to moderate (significant at day 56 while not significant at day 35) which are in accordance with our previous studies that the heritabilities of resistance to *V. harveyi* by challenge testing were also at low to moderate level in tongue sole (Li et al., 2019). In theory, genetic variation between families is expected to be at a maximum when cumulative mortality is approximate 50% (Gjerde et al., 2009), however, in our results, when cumulative mortality increased from 49.2% (day 35) to 71.6% (final mortality at day 56), family mortality variance increased from 0.0109 to 0.0134. This may explain why heritability estimates at day 56 were larger than at day 35.

Ideally, the genetic correlation between challenge test survival and natural outbreak survival with the same pathogen infection should be substantial, for example the case studies in Atlantic salmon, the genetic correlation between *Areomonas salmonicida* challenge test survival and field outbreak survival was 0.95 (Gjøen et al., 1997), and the genetic correlations between infectious pancreatic necrosis challenge tests and field outbreaks were also high (range 0.78–0.83) (Wetten et al., 2007). However, the genetic correlation between resistance to vibriosis in natural outbreak and resistance to *V. harveyi* by challenge test is very low and not significantly different from 0 (0.06 ± 0.05). This should be mainly ascribed to the co-infections of different *Vibrio* spp. Moreover, the challenge test was later than natural outbreak, actually, the population used in challenge test also undergone a natural outbreak (overall mortality was 33% with antibiotic treatments) before tagging.

When analyzing survival data from challenge tests or natural (field) outbreaks in aquaculture species, resistance is usually defined as continuous trait (test day, e.g., Yáñez et al., 2013), binary trait (dead/alive, e.g., Ødegård et al., 2006), binary survival scores trait (binary test day, e.g., Ødegård et al., 2007) or categorical trait (dead/infected/healthy, e.g., Bangera et al., 2014), then these trait definitions were fitted in each corresponding appropriate model. The agreement between genetic predictions of different methods was assessed based on the Pearson and Spearman rank correlation coefficients between full-sib family EBVs (mid-parents EBVs) for each model. The Pearson and Spearman correlation coefficients between full-sib family EBVs analyzed with different models are usually very high (> 0.90) across studies (Li et al., 2019; Liang et al., 2017; Yáñez et al., 2013; Bangera et al., 2014; Ødegård et al., 2006, 2007, 2010b). These results are accordance with this study (Spearman rank correlation coefficients > 0.98).

The selection accuracy of different models was commonly predicted based on Pearson correlation coefficients between the full-sib family EBVs obtained from each subset (parallel testing batches or randomly partitioned subsets), and the accuracy of selection varied across models and studies, ranging from 0.67 to 0.97 (Li et al., 2020a; Bangera et al., 2014; Sukhavachana et al., 2019; Yáñez et al., 2013; Ødegård et al., 2010b; Gitterle et al., 2006). But there is a clear trend that models with traits taken time till death into account (i.e., test day and binary test day) showed higher predictive ability. In this study, LRM with binary test day survival showed a slightly higher accuracy than other models.

Natural survival is a desirable trait and selective breeding based on this trait will benefit farmers directly. Precisely because of this, selective breeding by using natural outbreak survival data is encouraged, though we do not expect to suffer filed outbreak every year. Field outbreak data will be a very good data for the particular year-class. By and large, the selection will continue using disease challenge test results and aim should be to achieve a challenge test models as close as natural outbreak for getting a good genetic correlation between disease outbreak and challenge test. In this case study in tongue sole, we emphasize on the importance of using natural outbreak survival data as a novel approach for disease resistant breeding.

5. Conclusion

This is the first genetic analysis of vibriosis natural outbreak data in tongue sole. The estimated heritabilities for resistance to vibriosis using four different statistical models were low or not significantly different from zero at 35 days and were significant at day 56, indicating potential prospects for genetic improvement by using such data. Spearman rank correlations between family EBVs for the different models were all close to unity. Compare to simpler cross-section models (i.e., CLM, CTMl, CTMp), longitudinal model (i.e., LRM) integrated with time until death demonstrated the highest accuracy of family selection. Therefore, our results provided a novel perspective for the selective breeding of disease resistance in tongue sole.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the China Agriculture Research System (CARS-47-G03).

References

Azéma, P., Lamy, J.B., Boudry, P., Renault, P., Travers, M.A., Dégremont, L., 2017. Genetic parameters of resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific oyster, *Crassostrea gigas*, at three different life stages. Genet. Sel. Evol. 49, 23.

Bangera, R., Ødegård, J., Præbel, A.K., Mortensen, A., Nielsen, H.M., 2011. Genetic correlations between growth rate and resistance to vibriosis and viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). Aquaculture 317, 67–73.

Bangera, R., Ødegård, J., Nielsen, H.M., Gjøen, H.M., Mortensen, A., 2013. Genetic analysis of vibriosis and viral nervous necrosis resistance in Atlantic cod (*Gadus morhua* L.) using a cure model. J. Anim. Sci. 91, 3574–3582.

Bangera, R., Ødegård, J., Mikkelsen, H., Nielsen, H.M., Seppola, M., Puvanendran, V., Gjøen, H.M., Hansen, Ø.J., Mortensen, A., 2014. Genetic analysis of francisellosis field outbreak in Atlantic cod (*Gadus morhua* L.) using an ordinal threshold model. Aquaculture 420–421, S50–S56.

Butler, D.G., Cullis, B.R., Gilmour, A.R., Gogel, B.G., Thompson, R., 2017. ASReml-R Reference Manual Version 4. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Cabello, F.C., Godfrey, H.P., Tomova, A., Ivanova, L., Dolz, H., Millanao, A., Buschmann, A.H., 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ. Microbiol. 15, 1917–1942.

Cano-Gomez, A., Høj, L., Owens, L., Baillie, B.K., Andreakis, N., 2015. A multiplex PCR-based protocol for identification and quantification of *Vibrio harveyi*-related species. Aquaculture 437, 195–200.

Cox, D.R., 1972. Regression models and life-tables. J. Roy. Stat. Soc. B Met. 34, 187–220.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, Fourth ed. Longman Group, Essex, UK (464 pp).

FAO, 2015. The Impact of Natural Hazards and Disasters on Agriculture and Food Security and Nutrition: A Call for Action to Build Resilient Livelihoods. www.fao.org/3/a-i4434e.pdf.

FAO, 2016. The State of World Fisheries and Aquaculture 2016. Contributing to Food Security and Nutrition for all. Rome. 200 pp. www.fao.org/3/a-i5555e.pdf.

Gauger, E., Smolowitz, R., Uhlinger, K., Casey, J., Gómez-Chiarri, M., 2006. *Vibrio harveyi* and other bacterial pathogens in cultured summer flounder *Paralichthys dentatus*. Aquaculture 260, 10–20.

Gitterle, T., Ødegård, J., Gjerde, B., Rye, M., Salte, R., 2006. Genetic parameters and accuracy of selection for resistance to White Spot Syndrome Virus (WSSV) in *Penaeus* (*Litopenaeus*) *vannamei* using different statistical models. Aquaculture 251, 210–218.

Gjerde, B., Evensen, Ø., Bentsen, H.B., Storset, A., 2009. Genetic (co)variation of vaccine injuries and innate resistance to furunculosis (*Aeromonas salmonicida*) and infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*). Aquaculture 287, 52–58.

Gjøen, H.M., Refstie, T., Ulla, O., Gjerde, B., 1997. Genetic correlations between survival of Atlantic salmon in challenge and field tests. Aquaculture 158, 277–288.

Guan, C., Ding, Y., Ma, A., Wang, Y., Li, J., Ni, Q., Liu, X., Wang, Q., Mai, K., Lin, H., Huang, B., Yang, Z., 2018. Flatfish farming. In: Gui, J., Tang, Q., Li, Z., Liu, J., De Silva, S.S. (Eds.), Aquaculture in China, pp. 309–328.

Huang, Y., Yin, Z., Weng, S., He, J., Li, S., 2012. Selective breeding and preliminary commercial performance of *Penaeus vannamei* for resistance to white spot syndrome virus (WSSV). Aquaculture 364–365, 111–117.

Kaplan, E.L., Meier, P., 1958. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 457–481.

Kettunen, A., Fjalestad, K.T., 2006. Resistance to vibriosis in Atlantic cod (*Gadus morhua* L.): first challenge test results. Aquaculture 258, 263–269.

Kettunen, A., Serenius, T., Fjalestad, K.T., 2007. Three statistical approaches for genetic analysis of disease resistance to vibriosis in Atlantic cod (*Gadus morhua* L.). J. Anim. Sci. 85, 305–313.

Kim, M.S., Cho, J.Y., Choi, H.S., 2014. Identifcation of *Vibrio harveyi*, *Vibrio ichthyoenteri* and *Photobacterium damselae* isolated from olive flounder *Paralichthys olivaceus* in Korea by multiplex PCR developed using the *rpo*B gene. Fish. Sci. 80, 333–339.

Leeds, T.D., Silverstein, J.T., Weber, G.M., Vallejo, R.L., Palti, Y., Rexroad III, C.E., Evenhuis, J., Hadidi, S., Welsh, T.J., Wiens, G.D., 2010. Response to selection for bacterial cold water disease resistance in rainbow trout. J. Anim. Sci. 88, 1936–1946.

Li, Y., Wang, L., Lu, S., Wang, S., Zhang, H., Yang, Y., Li, M., Chen, S., 2020a. Heritability of disease resistance to *Edwardsiella tarda* in olive flounder (*Paralichthys olivaceus*). Aquaculture 519, 734750.

Li, Y., Wang, L., Yang, Y., Li, X., Dai, H., Chen, S., 2019. Genetic analysis of disease resistance to *Vibrio harveyi* by challenge test in Chinese tongue sole (*Cynoglossus semilaevis*). Aquaculture 503, 430–435.

Li, M., Yang, Y., Zheng, W., Li, Z., Cheng, J., Li, Y., 2020b. Estimation of heritabilities of disease resistance to *Edwardsiella tarda* and genetic correlations between resistance and growth traits in Chinese tongue sole (*Cynoglossus semilaevis*). Aquacult. Fish. https://doi.org/10.1016/j.aaf.2019.12.002.

Liang, B., Jiang, F., Zhang, S., Yue, X., Wang, H., Liu, B., 2017. Genetic variation in vibrio resistance in the clam *Meretrix petechialis* under the challenge of *Vibrio parahaemolyticus*. Aquaculture 468, 458–463.

Liu, L., Ge, M., Zheng, X., Tao, Z., Zhou, S., Wang, G., 2016a. Investigation of *Vibrio alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* in large yellow croaker, *Pseudosciaena crocea* (Richardson) reared in Xiangshan Bay. China. Aquac. Rep. 3, 220–224.

Liu, F., Li, Y.Z., Wang, X.X., Liu, X.F., Xing, H.F., Wu, Y.H., Xiu, W.S., Shao, C.W., Chen, S.L., 2016b. Estimation of genetic parameters for disease-resistance traits in *Cynoglossus semilaevis* (Günther, 1873). J. Appl. Ichthyol. 32, 643–651.

Magnadottir, B., 2010. Immunological control of fish diseases. Mar. Biotechnol. 12, 361–379.

Mohamad, N., Amal, M.N.A., Yasin, I.S.M., Saad, M.Z., Nasruddin, N.S., Al-saari, N., Mino, S., Sawabe, T., 2019. Vibriosis in cultured marine fishes: a review. Aquaculture 512, 734289.

Nguyen, H.T., Nguyen, T.T.T., Tsai, M.A., Ya-Zhen, E., Wang, P.C., Chen, S.C., 2017. A formalin-inactivated vaccine provides good protection against *Vibrio harveyi* infection in orange-spotted grouper (*Epinephelus coioides*). Fish Shellfish Immunol. 65, 118–126.

Ødegård, J., Olesen, I., Gjerde, B., Klemetsdal, G., 2006. Evaluation of statistical models for genetic analysis of challenge test data on furunculosis resistance in Atlantic salmon (*Salmo salar*): prediction of field survival. Aquaculture 259, 116–123.

Ødegård, J., Olesen, I., Gjerde, B., Klemetsdal, G., 2007. Evaluation of statistical models for genetic analysis of challenge-test data on ISA resistance in Atlantic salmon (*Salmo salar*): prediction of progeny survival. Aquaculture 266, 70–76.

Ødegård, J., Meuwissen, T., Heringstad, B., Madsen, P., 2010a. A simple algorithm to estimate genetic variance in an animal threshold model using Bayesian inference. Genet. Sel. Evol. 42, 29.

Ødegård, J., Sommer, A.I., Prabel, A.K., 2010b. Heritability of resistance to viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). Aquaculture 300, 59–64.

Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. Aquac. Res. 42, 103–114.

Rao, B.M., Lalitha, K.V., 2015. Bacteriophage for aquaculture: are they beneficial or inimical. Aquaculture 437, 146–154.

Song, S.K., Beck, B.R., Kim, D., Park, J., Kim, J., Kim, H.D., Ringø, E., 2014. Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol. 40, 40–48.

Song, Y., Zheng, W., Zhang, M., Cheng, X., Cheng, J., Wang, W., Zhang, J., Li, Y., 2020. Out-of-season artificial reproduction techniques of cultured female tongue sole (*Cynoglossus semilaevis*): broodstock management, administration methods of hormone therapy and artificial fertilization. Aquaculture 518, 734866.

Sukhavachana, A., Poompuang, S., Poompuang, S., Luengnaruemitchai, A., 2019. Heritability estimates and selection response for resistance to *Streptococcus agalactiae* in red tilapia *Oreochromis* spp. Aquaculture 502, 384–390.

Tang, X.Q., Zhou, L., Zhan, W.B., 2008. Isolation and characterization of pathogenic *Listonella anguillarum* of diseased half-smooth tongue sole (*Cynoglossus semilaevis* Günther). J. Ocean U China 7, 343–351.

Wang, X., Ma, A., 2019. Genetic parameters for resistance against *Vibrio anguillarum* in turbot *Scophthalmus maximus*. J. Fish Dis. 42, 713–720.

Wetten, M., Aasmundstad, T., Kjøglum, S., Storset, A., 2007. Genetic analysis of resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). Aquaculture 272, 111–117.

Yáñez, J.M., Bangera, R., Lhorente, J.P., Oyarzún, M., Neira, R., 2013. Quantitative genetic variation of resistance against *Piscirickettsia salmonis* in Atlantic salmon (*Salmo salar*). Aquaculture 414–415, 155–159.

Yáñez, J.M., Houston, R.D., Newman, S., 2014. Genetics and genomics of disease resistance in salmonid species. Front. Genet. 5, 415.

Zhang, X., Wang, S., Chen, S., Chen, Y., Liu, Y., Shao, C., Wang, Q., Lu, Y., Gong, G., Ding, S., Sha, Z., 2015. Transcriptome analysis revealed changes of multiple genes involved in immunity in *Cynoglossus semilaevis* during *Vibrio anguillarum* infection. Fish Shellfish Immunol. 43, 209–218.