Accuracies of genomic predictions for disease resistance of striped catfish to *Edwardsiella ictaluri* using artificial intelligence algorithms

Nguyen Thanh Vu[a,b,c,*], Tran Huu Phuc[c], Kim Thi Phuong Oanh[d], Nguyen Van Sang[c], Trinh Thi Trang[a,b,e], Nguyen Hong Nguyen[a,b,*]

[a]Genecology Research Center, [b]School of Science, Technology and Engineering, University of the Sunshine Coast, 90 Sippy Downs drive, Sippy Downs, QLD 4556, Australia; [c]Research Institute for Aquaculture No.2, 116 Nguyen Dinh Chieu, District 1, Ho Chi Minh city, Vietnam; [d]Institute of Genome Research, Vietnam Academy of Science and Technology, Cau Giay, Hanoi, Vietnam; [e]Vietnam National University of Agriculture, Gia Lam, Vietnam.

*Correspondence to N.T. Vu vunt.ria2@mard.gov.vn or ThanhVu.Nguyen@research.usc.edu.au and N.H. Nguyen nnguyen@usc.edu.au

Abstract

Assessments of genomic prediction accuracies using artificial intelligence (AI) algorithms (i.e., machine and deep learning methods) are currently not available or very limited in aquaculture species. The principal aim of this study was to examine the predictive performance of these new methods for disease resistance to *Edwardsiella ictaluri* in a population of striped catfish *Pangasianodon hypophthalmus* and to make comparisons with four common methods, i.e., pedigree-based best linear unbiased prediction (PBLUP), genomic-based best linear unbiased prediction (GBLUP), single-step GBLUP (ssGBLUP) and a non-linear Bayesian approach (notably BayesR). Our analyses using machine learning (i.e., ML-KAML) and deep learning (i.e., DL-MLP and DL-CNN) together with the four common methods (PBLUP, GBLUP,

ssGBLUP and BayesR) were conducted for two main disease resistance traits (i.e., survival status coded as 0 and 1 and survival time, i.e., days that the animals were still alive after the challenge test) in a pedigree consisting of 560 individual animals (490 offspring and 70 parents) genotyped for 14,154 Single Nucleotide Polymorphism (SNPs). The results using 6470 SNPs after quality control showed that AI methods outperformed PBLUP, GBLUP and ssGBLUP, with the increases in the prediction accuracies for both traits by 9.1 – 15.4%. However, the prediction accuracies obtained from AI methods were comparable to those estimated using BayesR. Imputation of missing genotypes using AlphaFamImpute increased the prediction accuracies by 5.3 – 19.2% in all the methods and data used. On the other hand, there were insignificant decreases (0.3 – 5.6%) in the prediction accuracies for both survival status and survival time when multivariate models were used in comparison to univariate analyses. Interestingly, the genomic prediction accuracies based on only highly significant SNPs ($P < 0.00001$, 318 - 400 SNPs for survival status and 1362 – 1589 SNPs for survival time) were somewhat lower (0.3 to 15.6%) than those obtained from the whole set of 6,470 SNPs. In most of our analyses, the accuracies of genomic prediction were somewhat higher for survival time than survival status (0/1 data). It is concluded that there are prospects for the application of genomic selection to increase disease resistance to *Edwardsiella ictaluri* in striped catfish breeding programs.

1. Introduction

Genomic selection has been increasingly practiced in genetic improvement programs for farmed animals and plants, using a range of different statistical methods from genomic-based best linear unbiased prediction (GBLUP) and its extension known as single-step GBLUP (ssGBLUP) to non-linear Bayesian approaches (BayesA, BayesB, BayesC, BayesC-π and notably BayesR) (Lourenco et al. 2020; Moser et al. 2015; VanRaden 2008; Daetwyler et al. 2013). Recently, there has been a growing interest in using

46 Artificial Intelligent (AI) algorithms (i.e., Machine Learning or Deep Learning) to choose optimal genome-
47 wide models without prior consumptions to determine genomic prediction accuracies for quantitative
48 complex traits, especially for disease resistance (tolerance or resilience). Examples using simulated and
49 real animal and plant data showed that the prediction accuracies (*r*) using machine learning were 10%
50 greater than linear (i.e. GBLUP) and 1.3% greater than non-linear methods (i.e. BayesR), for instance, *r* =
51 0.732 – 0.758 for binary and continuous traits using GBLUP vs 0.801 – 0.832 (Yin et al. 2020) or the
52 increase of 20 – 70% in accuracy when using machine learning (i.e., linear bagging) for disease resistance
53 to photobacterium in gilthead sea bream (Bargelloni et al. 2021). To date, however, no (or very limited)
54 studies have used simultaneously AI methods to estimate genomic breeding values for aquaculture
55 species.

56 Almost all studies in aquatic animal species have employed GBLUP, ssGBLUP or Bayesian methods to
57 examine genomic prediction accuracies for two main groups of traits, i.e., body weight and disease
58 resistance. A synthesis of the published information shows that the genomic prediction accuracy ranged
59 from 0.38 to 0.89 for growth-related traits (Houston et al. 2020). On the other hand, the predictions of
60 genomic breeding values for disease resistance were varied with studies, pathogens, populations and
61 methods used, with the estimates ranging from 0 (Silva et al. 2019) to 0.8 or greater (Barría et al. 2018).
62 For both growth and disease traits, the majority of the studies demonstrated that either linear (GBLUP,
63 ssGBLUP) or non-linear Bayesian methods increased the prediction accuracies of animal breeding values
64 relative to Pedigreed-based BLUP (PBLUP) by 22 - 24% (Houston et al. 2020). In addition to growth and
65 disease traits, three recent studies performed genomic predictions for meat quality in banana shrimp
66 (Nguyen et al. 2020) or Portuguese Oyster (*Crassostrea angulata*) (Vu et al. 2021) as well as behavior
67 traits (i.e., cannibalism) in Asian seabass (Nguyen and Khang 2021).

68 Despite the economic importance of the striped catfish in aquaculture, e.g., valuing 2.36 billion USD that
69 accounts for 1% GDP of Vietnam, there has been a paucity of knowledge in using genomic information in

70 breeding programs for high growth (Vu et al. 2019b) or increased disease resistance, especially Bacillary
71 Necrosis of Pangasius (Vu et al. 2019a). The Bacillary Necrosis of Pangasius (BNP) due to *Edwardsiella*
72 *ictaluri* has been the main contributor to 56 – 92% mortality during larval and fingerling rearing in this
73 species (Vũ et al. 2017). Our earlier studies showed that there is heritable genetic component for *E.*
74 *ictaluri* resistance, but the heritability for this trait was low, around 0.10 across statistical methods used
75 (Vu et al. 2019a; Pham et al. 2021; Dinh Pham et al. 2021). While these results suggests there are
76 prospects for genetic improvement of resistance to *E. ictaluri* using conventional selective breeding, the
77 pathogen test has posed substantial challenges in terms of biosecurity issues, times, costs and
78 environmental impacts (Pham et al. 2020; Nguyen 2014). Due to these limitations, genomic selection
79 has emerged as an alternative option to increase resistance of striped catfish to *E. ictaluri*, one of the
80 most severe diseases that has caused significant economic loss for the sector world-wide.

81 Therefore, the principal of this study was to assess genomic prediction accuracies of machine and deep
82 learning methods for two main disease resistance traits (i.e., survival status and survival time) and to
83 make comparisons with four common methods (PBLUP, GBLUP, ssGBLUP and BayesR). Additionally, we
84 explored if imputation of missing genotypes, multiple traits analyses and significant genome-wide
85 markers can improve the prediction accuracies for the disease resistant traits. Our results open new
86 opportunities for genome-based selection to increase the animal resistance to *E. ictaluri* in striped
87 catfish as well as other aquaculture species infected by this highly infectious pathogen.

88 **2. Materials and Methods**

89 **Ethical statement**

90 All the methods and experimental protocols of this study were performed in accordance with guidelines
91 and regulations approved by the animal ethics committee of the University of the Sunshine Coast,
92 Australia (approval number ANE1826).

93 **2.1. Fish and challenge test**

94 The animal samples used in this study originated from a selective breeding program for improved
95 disease resistance of striped catfish to *E. ictaluri* (Vu et al. 2019a). In 2020, the first generation was
96 produced based on a nested mating design with a ratio of one male to two females. A total of 166
97 families (32 full- and 134 half-sib families) were successfully produced following the breeding protocol as
98 detailed in Van Sang et al. (2012) and Vu et al. (2019b). Fry of each family were kept in separate fiber
99 glass tanks up to 3 weeks before they were transferred to stock in net hapa installed in earthen pond.
100 When the fingerlings reached an average body weight of 15 – 20 g, a random sample of 100 fish per
101 family was individually identified using PIT (Passive Integrated Transponder) tag. After tagging, a half of
102 each family was sent to ponds for performance testing and another half was used in pathogen challenge
103 tests for *E. ictaluri* resistance.

104 The challenge test involved a total of 5328 individuals from 166 families (averaging 32 individuals per
105 family). The experimental fish were initially acclimatized in cement tanks for about two weeks. Then the
106 same number of fish from each family was randomly allocated to six cement tanks (10 m^3) for the
107 challenge test using cohabitant method (Vu et al. 2019a). The cohabitant fish (16.7 ± 6.1 g) were firstly
108 inoculated with the bacteria *E. ictaluri* pathogen (10^6 CFU/0.2 ml per fish). Two days after the injection,
109 they were released into the cement tanks to rear with the experimental fish with a ratio of 1 to 3 (or
110 roughly 30% cohabitant fish in each tank). The experiment was conducted over a period of 23 days.
111 During this period, the feeding rate was reduced to 1.5% of the total biomass in tank. Mortality was
112 highest in day 5 and dead fish were sampled for laboratory PCR test to verify that their death symptoms
113 (white spots in spleen, liver and kidney) were due to *E. ictaluri* pathogen. At the conclusion of the
114 experiment, all alive fish were biosecure-buried, following the regulations of the national veterinary
115 authority (Department of Animal Health, Vietnam).

116 **2.2. Phenotype data**

117 During the challenge test, death fish were collected every 3 hours and their clinical symptoms were also
118 recorded. The data were used to calculate two measures of *E. ictaluri* resistance, i.e., survival status and
119 survival time. Survival status was expressed as a binary trait in which dead fish were designated as zero
120 (0) and alive animals at the end of the challenge test were assigned a number 1. Furthermore, survival
121 time was defined as the continuous trait from the start of test until the animal death in day. Both
122 survival status and survival time were analyzed using linear mixed model by ASReml 4.1 (Gilmour et al.
123 2014) to estimate breeding values (EBVs) for all individuals (5,328) and families (166) in the pedigree,
124 with common environmental effect – c^2 (i.e., accounting for differences due to separate rearing of
125 families until tagging) fitted in the model. Based on the EBVs ranking, 20 highest resistance families and
126 20 lowest resistance families were chosen from 166 families. Only families with the number of offspring
127 greater than 9 were selected to ensure that the EBVs were estimated with a high level of reliability.
128 Next, we randomly collected fin tissue samples of 12 – 15 fish per family per disease resistance group for
129 genome sequencing using Diversity Arrays Technology (DArTseq™).

130 **2.3. Genotyping and quality control**

131 A total of 564 DNA samples (from 564 individual fish) were sent to a commercial service provider in
132 Canberra, Australia for genotyping by sequencing using DArTseq™ technology. The DArTseq™ was
133 based on the genome complexity reduction method in combination with high throughput next
134 generation sequencing using Illumina platform. The sequencing protocol including choice of restricted
135 enzymes and library preparation was optimized for striped catfish (Pangasianodon DarTseq 1.0) using 96
136 independent DNA samples of striped catfish in our earlier study (Vu et al. 2020). Briefly, the PstI-SphI
137 method (Kilian et al. 2012) was used and the compatible adaptors were designed to include Illumina
138 flow-cell attachment sequence, sequencing primer sequence and capturing variant length of barcode

139 regions (Elshire et al. 2011). Then, only mixed fragments (PstI-SphI) were amplified in 30 rounds of PCR,
140 followed by sequencing on Illumina Hiseq2500 (77 cycles per single read). Next, sequences generated in
141 each plate with 96-well microliter were processed by proprietary DArT analysis protocol. The genotype
142 calling were partially described in our previous studies (Nguyen et al. 2020; Nguyen et al. 2018). The
143 average variant call-rate was 99% and the sample calling rate was 92%. With this quality control, four
144 out of 564 samples were discarded, and 560 samples (490 offspring and 70 parents) and 14.154 SNPs
145 remained for further quality control by dartR package (Gruber et al. 2018). In this data, average read
146 depth in each locus ranged 2,800 to 122,106 sequences, reads count from 1,410 to 81,949 sequences.
147 The quality control of the genotype data in the dartR package (Gruber et al. 2018) was filtered for loci
148 call-rate (<0.05, 4665 SNPs removed) and individual call-rate (<0.9, 2 individuals removed),
149 monomorphic SNPs (0 SNP removed), minor allele frequency (<0.05, 2830 SNPs removed) and significant
150 SNPs departure from Hardy-Weinberg Equilibrium (<0.05, 0 SNP removed). The retaining 6659 SNPs
151 were blasted to the striped catfish genome database GENO_Phyp_1.0 (Kim et al. 2018) which matched
152 6470 SNPs to the genome chromosome information. The genotype data of 6470 SNPs and the
153 phenotypes of 488 individuals were used for subsequent analyses.

154 **2.4. Genotype imputation**

155 The missing genotypes were about 10.0% in this study. They were imputed using AlphaFamImpute
156 (Whalen et al. 2020). The imputation was based on offspring-parent (i.e., pedigree) information.
157 Specifically, this method used parental genotypes to impute the missing values of offspring genotypes
158 and then, the offspring genotypes were used to fulfill the missing values of their parents (Whalen et al.
159 2020). The pedigree used in our analysis was traced back to the base population, including 4
160 generations.

161 **2.5. Estimation of heritability using genotype data**

162 Variances components of the two studied traits (survival status and survival time) were estimated using
163 linear mixed model under the Best Linear Unbiased Prediction (BLUP) framework in AIREMLf90 sub-
164 program of the BLUPF90 family package (Misztal et al. 2002). AIREMLf90 uses Average Information-
165 Restricted Maximum Likelihood method that requires less computational resources and higher accuracy
166 of variance estimates (Masuda et al. 2014). The models included the fixed effects of spawning batch (4
167 levels, 1 – 2 weeks interval between successive spawning batches) and pathogen challenge test tanks (2
168 levels) and the random effect of additive genetics of the individual fish in the pedigree. Age from birth to
169 tagging (124 – 167 days) was also fitted as a linear covariate. The SNP heritability (h^2) was calculated as
170 a ratio of the additive genetic variance (σ_a^2) to total phenotypic variance (σ_p^2), where σ_p^2=σ_a^2+σ_e^2 (σ_e^2 =
171 environmental variance). Based on the logarithmic likelihood ratio test (LRT), the common
172 environmental effect (c^2) was not significant (P > 0.05) and hence, this effect was omitted from the
173 statistical models used to estimate genetic variances and prediction accuracy.

174 **2.6. Statistical methods**

175 Accuracies of genomic prediction for the disease resistance traits (survival status and survival time) were
176 estimated using BLUP-family methods (PBLUP, GBLUP, ssBLUP), BayesR, machine learning (i.e., KAML)
177 and deep learning (i.e., DeepGP).

178 **2.6.1. PBLUP, GBLUP and ssGBLUP (using BLUPF90 program)**

179 The mixed model used in PBLUP, GBLUP and ssGBLUP is written in a matrix notation as follows:

180 $$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Za} + \boldsymbol{e} \qquad \text{(equation 1)}$$

181 Where $\boldsymbol{y}$ is the vector of phenotypic values (survival status or survival time), $\boldsymbol{b}$ is the vector of fixed
182 effects (i.e., spawning batches and experimental tanks and a linear covariate of age from birth to
183 tagging), $\boldsymbol{a}$ is the vector of the random term (i.e., the additive genetic effect of individual fish in the

184 pedigree). ***X*** and ***Z*** are the incidence matrices related to the fixed and random effects. The letter ***e*** refers
185 to residual variance or error of the estimates corresponded to each phenotypic value.

186 The main difference among BLUP-family methods (PBLUP, GBLUP and ssGBLUP) relates to relationship
187 matrices (**A**, **G** or **H**) used to solve the mixed model equation 1 above.

188 **PBLUP:** In PBLUP (Henderson 1985), ***a*** is the additive genetic effect of each individual fish with its
189 corresponding matrix **Z** following a normal distribution ~ N(0, $\mathbf{A}\sigma_a^2$), with **A** is the numerator relationship
190 matrix calculated from the pedigree records and σ_a^2 is the additive genetic variance.

191 **GBLUP**: In GBLUP (Meuwissen et al. 2001), ***a*** is the random additive genetic effect underlying polygenic
192 effects of SNPs with a normal distribution ~ N(0, $\mathbf{G}\sigma_g^2$) where genomic relationship matrix **G** was
193 generated by VanRaden method (VanRaden 2008) from 6,470 SNPs and genomic variance σ_g^2. The
194 GBLUP method assumes that each SNP contributes little to phenotypic variance of the traits studied.

195 **ssGBLUP**: In ssGBLUP (Misztal et al. 2009; Lourenco et al. 2020), **A** and **G** matrices are blended to
196 produce realized matrix **H** (Aguilar et al. 2010). The inverse of H matrix is expressed as below:

197 $$\boldsymbol{H^{-1}} = \boldsymbol{A^{-1}} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{G^{-1}} - \boldsymbol{A_{22}^{-1}} \end{bmatrix} \qquad \text{(equation 2)}$$

198 Where $\mathbf{A}^{-1}$ and $\mathbf{G}^{-1}$ are the inverse of matrices **A** and **G** as described above. $\mathbf{A}^{-1}_{22}$ is the inverse of matrix of
199 genotyped individuals only.

200 The three BLUP methods (PBLUP, GBLUP and ssGBLUP) were implemented in BLUPF90 package (Misztal
201 et al. 2002). For survival status, we used generalized (threshold) mixed model in THRGIBBF1f90 (Tsuruta
202 and Misztal 2006) known as threshold Gibb Sampling method, whereas survival time was analyzed using
203 linear mixed models in AIREMLf90 (Masuda et al. 2014). In the Gibbs sampling, we used 200,000
204 iterations with 20,000 iterations as burn-in for univariate analysis. The convergence of the parameters
205 estimates was checked using POSTGIBBSf90 program.

206 **2.6.2. Non-linear Bayesian method (BayesR)**

207 BayesR assumes that SNP effects β_i follow a four-component normal mixture and the effect of SNP *i* is
208 assumed to be distributed as:

209 $$\beta_i \sim \pi_1(\beta_i = 0) + \pi_2 N(0.0001\sigma_g^2) + \pi_3 N(0, 0.001\sigma_g^2) + \pi_4 N(0, 0.01\sigma_g^2) \quad \text{(equation 3)}$$

210 Where σ_g^2 as defined above, σ_g^2 represents the total additive genetic variance (i.e., the cumulative
211 variance of all SNP effects) and $\pi = (\pi_1, \pi_2, \pi_3, \pi_4)$ the mixing proportions such that $\sum_{i=1}^{4} \pi_i = 1$. The
212 mixing proportions π are assumed to follow a Dirichlet prior, $\pi \sim Dirichlet\ (\alpha + \beta)$,
213 with α representing a vector of pseudo counts and β the cardinality of each component. In this work,
214 we used a flat Dirchlet distribution, with $\alpha = (1,1,1,1)$ for the prior. As suggested by Moser et al. (2015),
215 σ_g^2 is assumed to be a random variable following an Inverse—χ2 distribution.

216 The analysis of BayesR consisted of three mains steps. First, numeric coding genotypes of 560 animals
217 (including 490 offspring and 70 parents) obtained from DArT sequencing were converted to ATGC
218 format (i.e., biallelic variant). Second, the trimmed sequences of each variant were blasted to nucleotide
219 non-redundant striped catfish database using Blast2GO (Conesa et al. 2005), named GENO_Phyp_1.0
220 (Kim et al. 2018). This analysis matched 13,766 SNPs to 30 autosomal chromosomes. The remaining
221 SNPs (388 SNPs) were randomly assigned to 30 chromosomes (12 – 13 SNPs/chromosome). Third, PLINK
222 v1.9 (Purcell et al. 2007) that utilizes genotype and mapping files was used to generate a full dataset for
223 BayesR (https://github.com/syntheke/BayesR). The default running cycles of BayesR included 50,000
224 steps with 20,000 steps discarded as burn-in. We used predicted phenotypes (computed in AIREMLf90
225 using equation 1), as this package does not allow fitting covariates in the model.

226 **2.6.3. Machine learning and deep learning methods**

227 **KAML (Kinship Adjusted Multiple Loci)**

228 KAML is a machine learning-based method that incorporates cross-validation, multiple regression, grid
229 search and bisection algorithm to improve genomic prediction accuracy for complex quantitative traits
230 (Yin et al. 2020). KAML extends linear mixed model (equation 1) by incorporating quantitative trait
231 nucleotides - QTNs (e.g., traits controlled by genes with large and moderate effects) as covariates and a
232 SNP-weighted trait-specific kinship matrix as the (co)-variance assumption corresponding to the random
233 effect. The selections of QTNs and SNP weights are optimized by machine learning. In a matrix notation,
234 equation 1 becomes:

235 $\boldsymbol{Y = Xb + Qq + Za^* + e}$ with $\boldsymbol{a}^* \sim N(0, K_w\sigma_g^2)$, $\boldsymbol{e} \sim N(0, I\sigma_e^2)$ (equation 4)

236 Where σ_g^2 and σ_e^2 are as described above; $\boldsymbol{Q}$ is covariates matrix of QTNs detected throughout multiple
237 regression analyses; $\boldsymbol{q}$ is a vector of fixed effect of each QTN corresponding to $\boldsymbol{Q}$ and K_w is SNP-weighted
238 Kinship that is optimized by grid search and bisection procedure. The implementation of KAML included
239 two main steps, i.e., define $\boldsymbol{Q}$ and K_w and then predicting genomic breeding values of the validation set.
240 In the training step, KAML optimized parameters for determining QTNs and SNPs weights which used all
241 testing subset data provided throughout cross-validation. Next, in the prediction step, the optimized
242 parameters were used to predict genomic breeding values of each individual in validation set. A detailed
243 description of the step-wise procedures and the underlying algorithms of KAML is given in Yin et al.
244 (2020). In our analyses, we used PLINK (Purcell et al. 2007) to convert the genotype data into the right
245 format to be analyzed in KAML (https://github.com/YinLiLin/KAML) in R environment (R Core Team
246 2015). KAML utilized adjusted phenotypes (i.e., phenotypic y-hats) for testing sets and masked (i.e., 'NA'
247 values) phenotypes of validation sets. To define the best parameters (i.e., pseudo QTNs, the optimal
248 SNPs weights, the optimal Log value and the optimized kinship matrix) for mixed models of each testing
249 set, we used five-fold cross-validation with 100 replications (to obtain good model estimates).

250 **Deep learning – Multilayer Perceptron (DL-MLP)**

For deep learning analysis, we used DeepGP package (https://github.com/lauzingaretti/deepGP/). A detailed description of the theoretical framework is given in Zingaretti et al. (2020) and Pérez-Enciso and Zingaretti (2019). In our analyses, we used two different algorithms of deep learning: Multilayer perceptron and convolutional neural network.

Multilayer perceptron is fully connected networks consisting of an input layer, one or several hidden layers, and an output layer. In the context of genomic prediction, input layer receives SNPs data (i.e., 6,470 SNPs). Each of SNP information in the input layer is transferred its value to the first neuron of the first hidden layer by the Z function:

$$Z^{(k)} = b_{k-1} + W^{(k-1)} f^{(k-1)}(x) \qquad \text{(equation 5)}$$

Where W is weighted SNP information, $f(x)$ is non-linear function and b is bias (i.e., constant). In the case of genomic prediction, the input layer is SNPs genotype (i.e., input variables = number of SNPs) of individual fully connected to the first hidden layers. As a result, the value in the first neuron of the first hidden layer is the sum effect of Z function over 6,470 SNPs ($\sum_{i=1}^{6470} Z$). Likewise, information in the first hidden layer is the input for transferring its value to the second hidden layers and finally to the output layers. The significance step in obtaining best DL-MLP model is to define MLP structure network (i.e, number of hidden layer and neuron of each layer) and hyperparameters which related to equation 5. These parameters and their values in our study include learning rate (--lr 0.025 0.01), type of drop out (--dr 0 0.01), type of regularization (--reg 0.0001), activation function (--act linear tanh), number of hidden layer (--h 1 5), optimizer function (--optimizer Adam) or number of epochs (--epochs 50). These scripts provided in DeepGP (https://github.com/lauzingaretti/DeepGP) were used to obtain hyperparameters in our DL-MLP analyses.

Deep learning – Convolutional Neural Network (DL-CNN)

273 Convolutional neural network comprises of 3 types of layers: (1) convolutional layers, (2) fully connected
274 layers and (3) input layers. In the context of genomic prediction, first convolutional layer receives data of
275 6,470 SNPs and write it to the first layer using kernel (i.e., a definite number of SNPs is grouped or
276 combined). Then, the pooling step is responsible to reduce spatial size of SNPs data of the convolutional
277 layer into the second convolutional layer. There are two types of pooling such as max pooling and
278 average pooling corresponding to maximum values and mean values of SNPs portion covered by the
279 kernel. The process was repeated to make update value of the last convolutional layer. At the last
280 convolutional layer, data were flattened (i.e., convert data of pooled feature of the last convolutional
281 layer into single column feeding to fully connected layers). The fully connected layer works similarly the
282 MLP. The hyperparameters used in our study are size of kernel (--ks 3), learning rate (--lr 0.025 0.01),
283 dropout in the first layer (-dr1 0 0.01), dropout in the hidden layer (--dr2 0 0.1 0.2), number of pooling
284 size (--ps 1), number of convolutional layers (--ncov 1 3), number of strides (--ns 1), number of
285 convolutional operations (--nfilters 8). These hyperparameters are fine-tuned under DeepGP with grid
286 search provided in DeepGP package (Zingaretti et al. 2020). As DeepGP cannot handle missing values,
287 we used imputed genotype for all analyses.

288 **2.6.4. Analysis of highly significant SNPs and multi-trait mixed model**

289 **ssGWAS to select significant SNPs for prediction**

290 Single step genome-wide association analysis (ssGWAS) was accomplished in BLUPF90 suite using each
291 subset phenotype data for both disease resistance traits. We used preGSf90 and postGSf90 together
292 with BLUPF90 program to compute SNP effect and its P-values. SNPs panels were selected based on the
293 P-value < 0.0001 (the number of the selected SNPs sets were presented in Supplementary Table S2).
294 These SNP panels were used to test the predictive performance of six genomic prediction methods used
295 in our study.

296 **Multi-trait mixed model**

297 Multi-trait analyses are only available in BLUPF90. In this study, two studied traits were jointly analyzed
298 using PBLUP, GBLUP and ssGBLUP models in THRGIBBS1F90 (Tsuruta and Misztal 2006). This involves
299 using Gibbs Sampling with MCMC updates including 1,000,000 iterations with 100,000 burn-in steps.

300 **2.7. Five-fold cross validation**

301 The predictive performances of all seven methods (PBLUP, GBLUP, ssGBLUP, BayesR, ML-KAML, DL-MLP
302 and DL-CNN) used in this study were evaluated using repeated 5-fold cross validation. The procedure
303 involved a random partitioning of the full data into 5 subsets (each subset has 97 – 98 individuals
304 including representatives of all the families). The phenotypes of one subset were masked and GEBV/EBV
305 (Genomic/Estimated Breeding Value) of one set were predicted using the information from the other
306 four subsets. This means one phenotype was used once in the validation process of one repetition. Each
307 analysis was repeated 5 times, giving 25 validation sets in total and the correlation coefficient was
308 averaged over 25 values. To compute evaluation metric in each validation set, the GEBV/EBVs of the
309 predicted phenotypes were correlated to the actual phenotype. We chose Pearson correlation
310 coefficient (Benesty et al. 2009) as an evaluation metric to consistently compare the statistical models
311 used. Moreover, we computed Mathew correlation coefficient, MCC (Matthews 1975) for binary trait (
312 survival status) as MCC is giving a different look (indicate which predicted value is assigned to 0 or 1
313 case) for binary classification evaluation (Chicco and Jurman 2020). Specifically, we used
314 *PresenceAbsence* R package (Freeman and Moisen 2008) to produce 4x4 confusion matrix which
315 assigned GEBV/EBV values against the actual phenotypes for computing MCC (Supplementary file Table
316 S1 for additional information and reference). To obtain the consistency and comparable results among
317 tested software/packages, we used the same subsets of testing/validation data in BLUPF90 family
318 program, BayesR, ML-KAML, DL-MLP and DL-CNN.

319 The genomic prediction accuracy for survival status and survival time was calculated as $Accuracy =$

320 $\frac{r_{y,\hat{y}}}{\sqrt{h^2}}$; where $r_{y,\hat{y}}$ is the correlation between the predicted breeding values $(\hat{y})$ and actual

321 phenotypes(y).

322 To evaluate reliability (or potential biases) of the statistical methods, we computed RMSE (Root Mean

323 Square Error) and R^2 (coefficient of determination) based on $\hat{y}$ and y values. RMSE is the standard

324 deviation of residuals, also known as prediction error, while coefficient of determination is an indicator

325 of the goodness of fit of a statistical model. RMSE and R^2 were calculated as below:

326 $$RMSE = \sqrt{\frac{\sum_1^n(\hat{y}-y)^2}{n}}; R^2 = 1 - \frac{\sum_1^n(\hat{y}-y)^2}{\sum_1^n(y-\bar{y})^2}$$

327 Where $\bar{y}$ is the average value of *n* actual phenotypes in a testing set.

328 **Data availability**

329 Data are available via Figshare portal provided by the journal.

330 **3. Results**

331 **3.1. Data, variance component and heritability**

332 The average survival rate and survival time after the pathogen challenge test were 32.2% and 163.9

333 hours, respectively (Table 1). When exposed to the *E. ictaluri* pathogen, mortality occurred after 32.5

334 hours and the highest death rate was observed after 97.3 hours (Figure 1). There were significant

335 differences ($P < 0.0001$, Figure 1) in both survival rate (60.0% vs. 4.5%) and survival time (207.8 vs. 120.0

336 hours) between 20 highest and 20 lowest resistant families (Table 1). The variations in the disease

337 resistant traits among families were also significant (Supplementary Figures S1 & S2).

338 The heritabilities estimated for survival status and survival time using traditional PBLUP (0.65 – 0.71)

339 were greater than those obtained from (ss)GBLUP (0.44 – 0.46) but they were lower than those

340 estimated by BayesR (0.96 – 0.97) (Table 2). Across statistical models (methods) used, the estimates of
341 heritability for survival status were slightly higher than those obtained for survival time (Table 2).

342 **3.2. Accuracy of genomic prediction using original genotype (un-imputed) data**

343 Figure 2 presents the accuracies of genomic breeding values for survival status and survival time using
344 Machine learning (ML-KAML) and Deep Learning (DL-MLP and DL-CNN) together with four other
345 methods (PBLUP, GBLUP, ssGBLUP and BayesR). When the original genotype data (i.e., un-imputed data)
346 were used, the prediction accuracies from ML-KAML (0.67) was slightly higher than PBLUP (0.66) and
347 they both were higher than those calculated from GBLUP (0.55) and ssGBLUP (0.55) for survival status.
348 ML-KAML also outperformed BLUP methods for survival time (0.69 vs 0.59-0.65) (also see
349 Supplementary Table S1). Additionally, the difference between ML-KAML and BayesR in the prediction
350 accuracy was small (0.67 for survival status and 0.69 vs. 0.70 for survival time). For both traits, ssGBLUP
351 was slightly better GBLUP. Interestingly, both GBLUP and ssGBLUP did not improve the prediction
352 accuracies for both survival status and survival time as compared with conventional PBLUP method.

353 **3.3. Accuracy of genomic prediction using imputed genotype data**

354 Imputation of missing genotypes increased the accuracies of genomic prediction for both survival status
355 and survival time across the seven methods used (Figure 3). The improvements for survival status were
356 from 12.0% (ML-KAML) to 19.2% (GBLUP). For survival time, the increase in the prediction accuracy
357 when the imputed genotypes were used as compared with un-imputed data was largest for GBLUP
358 (10.0%), followed by ML-KAML (8.1%), ssGBLUP (7.1%) and BayesR (5.3%). Deep learning (DL) using
359 multilayer perceptron (DL-MLP) and convolutional neural network (DL-CNN) had lower prediction
360 accuracies for both traits (0.67 – 0.73) than ML-KAML (0.75) or BayesR (0.75-0.76) methods but they
361 (DL-MLP and DL-CNN) had higher prediction accuracies than BLUP methods (0.55 – 0.66)

362 (Supplementary Table S1). ML-KAML and BayesR methods also had least variation (Min – Max values of
363 accuracy in the testing set) compared to other methods (Figure 3).

364 **3.4. Univariate vs. multi-variate analysis**

365 Our multivariate analyses used PBLUP, GBLUP and ssGBLUP, and the prediction accuracies for both
366 survival status and survival time are shown in Table 3. There were slight decreases observed in the
367 prediction accuracy when the three BLUP methods were used to estimate genomic breeding values
368 (GEBV) in multivariate analyses relative to univariate model. The reduction in the prediction accuracies
369 was only 2.9 to 5.6% for survival time and 0.3 to 2.5% for survival status.

370 **3.5. Genomic prediction in combination with GWAS**

371 The prediction accuracies for the disease resistance traits that incorporated only significant SNPs
372 identified from genome-wide association analysis (GWAS) are given in Tables 4 and 5. In all the genotype
373 subsets used, the prediction accuracies were generally smaller to those of the full set of 6,470 SNPs.
374 Using smaller SNP panels selected from ssGWAS with $p<0.00001$ in this study scarified accuracies by 0.3
375 to 15.6%. For survival status the prediction accuracy was reduced from 4.3 to 6.0% for ML-KAML and
376 BLUP methods, but greater loss was observed for deep learning methods (11.2% for DL-CNN and 15.6%
377 for DL-MLP). The same trend was observed for survival time but the reduction in the prediction accuracy
378 was only 0.3 to 4.9%.

379 **3.6. Reliability (or potential biases) of the statistical methods**

380 The root mean square error (RMSE) and coefficient of determination (R^2) were computed for each
381 method and they are shown in Tables 3 and 4. In both traits (survival status and survival time) and in all
382 analyses (univariate and multi-trait or full genotype vs subsets), the machine learning (i.e., ML-KAML)
383 gave the least possible biases in the breeding values prediction, as evidenced by the low RMSE (0.36 –

384 0.37 for survival status and 3.10 for survival time) and high R^2 (0.35 – 0.39). BayesR also displayed similar
385 predictive reliability to ML-KAML (RMSE = 0.36 – 0.37 for survival status and 0.311 for survival time and
386 R^2 =0.34 – 0.39). Among BLUP family methods, there were no large differences in the reliability of the
387 genomic breeding value estimation between GBLUP and ssGBLUP (for instance with univariate model
388 using un-imputed genotype, RMSE = 0.41 and R^2 = 0.20 – 0.21). However, they were likely less reliable
389 than PBLUP (RMSE = 0.39 and R^2 = 0.30). The utilization of the imputed genotypes in GBLUP and
390 ssGBLUP methods reduced the prediction errors for both survival status and survival time (RMSE = 0.39
391 and 3.31, and R^2 = 0.29 and 0.26, respectively).

392 **4. Discussion**

393 Development of disease resistance lines is essential for the aquaculture sector due to the combined
394 effects of aquaculture intensifications and environmental changes. Our study demonstrated that
395 genomic selection can be practiced improving resistance to *Edwardsiella ictaluri* disease in striped
396 catfish populations, using a range of different statistical methods especially machine and deep learning,
397 and BayesR. Salient findings from our study are discussed as follows.

398 First, the genomic prediction accuracies for the disease resistance traits (survival status and survival
399 time) were substantially improved when machine (i.e., ML-KAML) and deep learning (DL-MLP, DL-CNN)
400 methods were used relative to BLUP-family methods (PBLUP, GBLUP, ssGBLUP). In addition, our
401 evaluation of the prediction biases or prediction errors based on RMSE and R^2 statistics indicated that
402 the AI methods may be prone to the least errors in the estimated breeding values than BLUP family
403 methods. Hence, genomic selection to improve *E. ictaluri* disease is expected to achieve greater genetic
404 gain than the conventional selective breeding approach using pedigree and phenotype information. The
405 genomic selection can also shorten the breeding cycle of striped catfish as expected to reduce at least a
406 half of generation interval in dairy cattle (Hayes et al. 2009). Our results are consistent with those

407 reported in farmed animal (Baker et al. 2020), livestock (Li et al. 2018; González-Recio and Forni 2011)
408 and plants (Montesinos-López et al. 2019; Montesinos-López et al. 2018; Zingaretti et al. 2020). Despite
409 the superiority of the machine learning methods (i.e., ML-KAML) to PBLUP, GBLUP and ssGBLUP, they
410 had similar predictive power to BayesR for both traits in our study. Reports in farmed animals concluded
411 that BayesR is the best amongst Bayesian and GBLUP methods (Erbe et al. 2012) but no published
412 information is available in aquaculture species to compare with our study. To date, only some studies,
413 one in shrimp (Palaiokostas 2021) that used extreme gradient boost method (one of the machine
414 learning approaches) and another in seabream that used support vector machines and linear bagging
415 classification (Bargelloni et al. 2021) and reported that these methods improved the prediction accuracy
416 for survival traits by 1-4% and 20 – 70% relative to GBLUP and Bayesian methods. Recently, the
417 evaluation of deep learning model (e.g., convolutional neural network) in Bay scallop indicated this
418 method outperformed BayesB and random regression GBLUP methods (Zhu et al. 2021). Therefore,
419 machine or deep learning should be used for genomic evaluation of disease resistance traits, at least in
420 this population of striped catfish. The predictive powers of these methods using AI algorithms are
421 expected to be greater when big data (e.g., hundreds of thousands or million animals sequenced) are
422 analyzed for this population in the future.

423 Second, imputation of missing genotypes (using offspring and parent information available in the
424 pedigree of striped catfish) increased the predictive ability of all the seven methods used, especially
425 BayesR by 5.3% to approximately 19% (GBLUP and ssGBLUP) relative to when un-imputed data were
426 used. Also note that the imputation of missing genotypes in our study differed from others that used
427 software to compute missing genotypes without considering parental relationship. With the use of
428 offspring-parent information, the imputation likely increased accuracy of G and H matrices used in
429 GBLUP and single-step methods (i.e., ssGBLUP). Published information across species also showed that
430 imputation from low to high density SNP panels or from commercial SNP arrays to whole genome

431 sequence increased the genomic prediction accuracies for resistance to parasite (i.e. sea lice count) and
432 body traits in Atlantic salmon (Tsai et al. 2017), and agronomic traits in maize (Crossa et al. 2013).
433 However, some other studies also showed that imputed genotype did not improve prediction accuracies
434 in livestock animal breeding (Heidaritabar et al. 2016; Chen et al. 2014; Van Binsbergen et al. 2015).
435 Imputation has several benefits, firstly it can be made after sequencing with no extra cost. Second,
436 through imputation, we can increase the number of animals genotyped, which in turn increase selection
437 intensity, and this can lead to increases in genetic progress made in selected populations. Thus,
438 imputation of low-density sequence can support our future incorporation of genomic selection for
439 *Edwardsiella ictaluri* disease in this striped catfish population.

440 Third, we found that multivariate analysis did not improve the predictive ability for the two studied
441 traits irrespective of the statistical methods. These results are expected due to the high and positive
442 genetic correlations (r_g) between the two traits (r_g = 0.81). A bivariate analysis of harvest body weight
443 and fillet yield in Nile tilapia also showed no improvement in the prediction accuracies for these traits
444 (Joshi et al. 2020). Multi-trait analyses generally improve accuracy of genetic parameter estimates and
445 animal breeding values for traits whose genetic architectures are different (e.g., low vs high heritable) or
446 genetically antagonistic associated (i.e., unfavorably correlated). Examples of multivariate genomic
447 prediction models involving multi-trait in cassava and wheat plants gave better prediction accuracy over
448 univariate models (Okeke et al. 2017; Sun et al. 2017). To utilize the benefits of multi-trait analysis, we
449 continue collecting other traits of economic importance in striped catfish, namely disease tolerance,
450 disease resilience as well as immunological parameters to increase overall resistant capacity of the
451 animals in this catfish population.

452 Fourth, we also suggest using the full set of SNPs to obtain a reasonable level of prediction accuracy for
453 genomic selection to improve disease resistance traits in striped catfish. It has been well documented
454 that the prediction accuracy increased with number of markers and sequencing depth or genome

455 coverage. Due to characteristics of RAD-sequencing methods, there are still limited number of markers
456 in our study as compared with those reported in farmed animals or plants (Robledo et al. 2018; Davey
457 and Blaxter 2010). It is necessary to examine prediction ability of whole genome data when sequencing
458 costs are reduced to an affordable level to sequence thousands (or hundreds of thousands) of animals in
459 a near future. However, there are studies in farmed animals reporting that whole genome sequence
460 data improved the predictive powers for complex traits by only 0.4 – 5.0% (Al Kalaldeh et al. 2019;
461 VanRaden et al. 2017).

462 Finally, the utilization of highly significant SNPs from ssGWAS can give comparative prediction
463 accuracies for the two traits studied, for both un-imputed and imputed genotype data, as compared to
464 whole SNP panel. Using significant SNP panels selected from ssGWAS had higher accuracy in the
465 estimated breeding values for disease resistance traits in *Litopeneaus vannamei* (Luo et al. 2021) or fish
466 species (Jeong et al. 2020). On the one hand, these SNPs can be used to develop a small SNP panel to
467 genotype a large number of animals in both the training and validation populations. On the other hand,
468 the costs of genotyping animals for hundreds of SNPs may not be a lot cheaper than DArT sequencing as
469 used in this study. The use of significant SNPs can be beneficial when their biological functions
470 (pathways) are known. In species where reference genome or high quality genome assembly is available,
471 the incorporation of SNPs with biological functions (i.e. multi-omics) improved the prediction accuracy,
472 for instance, about 5 - 19% for fertility traits in dairy cattle (Abdollahi-Arpanahi et al. 2017; Nani et al.
473 2019), or 27.4 – 60.7% for traits in inbred lines of Drosophila (Ye et al. 2020). Unfortunately, a good
474 genome assembly is currently not available for striped catfish (Kim et al. 2018), this area thus deserves
475 future studies to enable the efficient utilization of genomic information in the genetic selection program
476 for this economically important species in the aquaculture sector.

477 In summary, despite the high level of consistency in the prediction accuracies across the seven methods
478 used, the estimates of heritability and prediction accuracies obtained for survival status and survival

479 time are potentially biased upward due to the family structure of the current population that included
480 selective genotypes (high and low families). However, this problem may have been alleviated because
481 both pedigree and genotype data were included in mixed models (e.g., BLUP family methods) to
482 estimate genomic breeding values (Gowane et al. 2019) or machine learning methods as demonstrated
483 in this study.

484 **5. Concluding remarks**

485 The genomic prediction accuracies for disease resistance traits of striped catfish were moderate to high,
486 suggesting possibilities for the application of genomic selection to improve resilience to *Edwardsiella*
487 *ictaluri* in this population. Machine learning and deep learning methods outperformed BLUP-family in
488 most of our analyses, regardless full or subset data or only significant SNPs used with imputed genotype.
489 However, the prediction accuracies using machine learning and deep learning for both survival status
490 and survival time were almost similar to those obtained from BayesR. There were no clear advantages of
491 machine learning and deep learning to conventional methods (PBLUP, GBLUP and ssGBLUP) if original
492 genotype was used for survival status but its superiority was evidenced for survival time. Therefore,
493 either machine learning, deep learning or BayesR could be used for genomic evaluation of the disease
494 resistance traits in this striped catfish population. Furthermore, breeding to improve resistance to *E.*
495 *ictaluri* can use survival status or survival time as alternative selection criterion as there was no
496 significant difference in the prediction accuracies across the seven different methods used in our study.
497 However, when more data are accumulated in future generations, it is necessary to re-evaluate the
498 prediction accuracies and potential biases before any practical implementation of genomic selection can
499 be made to improve disease resistance of this stripped catfish population.

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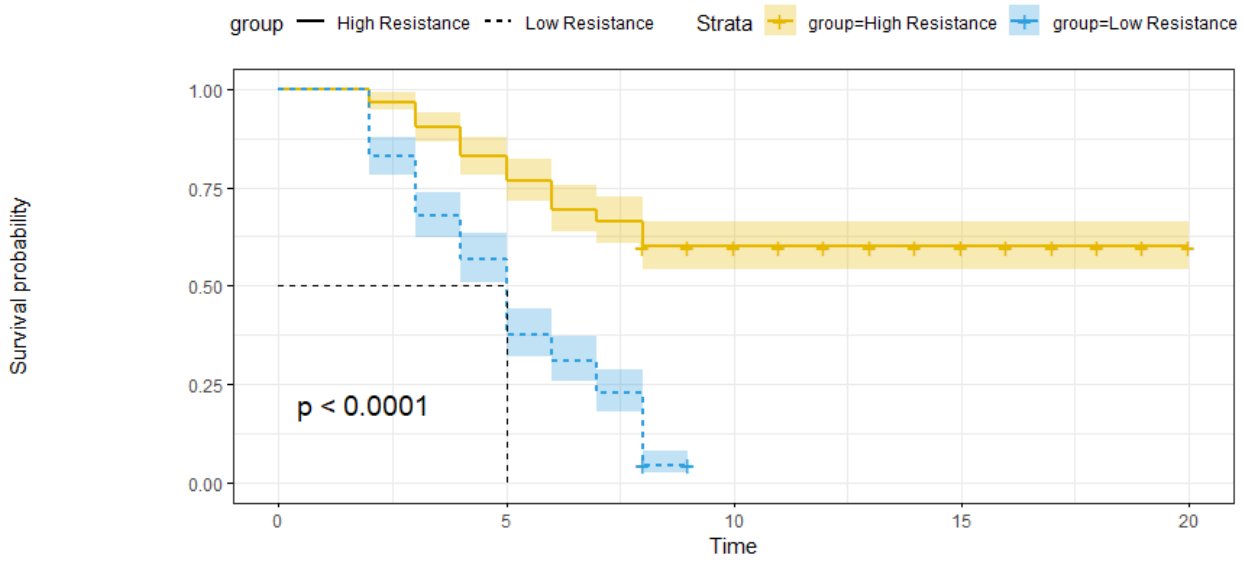

691

692 Figure 1. Survival trend in day post challenge of high and low resistance groups in genotyped individuals
693 of striped catfish.

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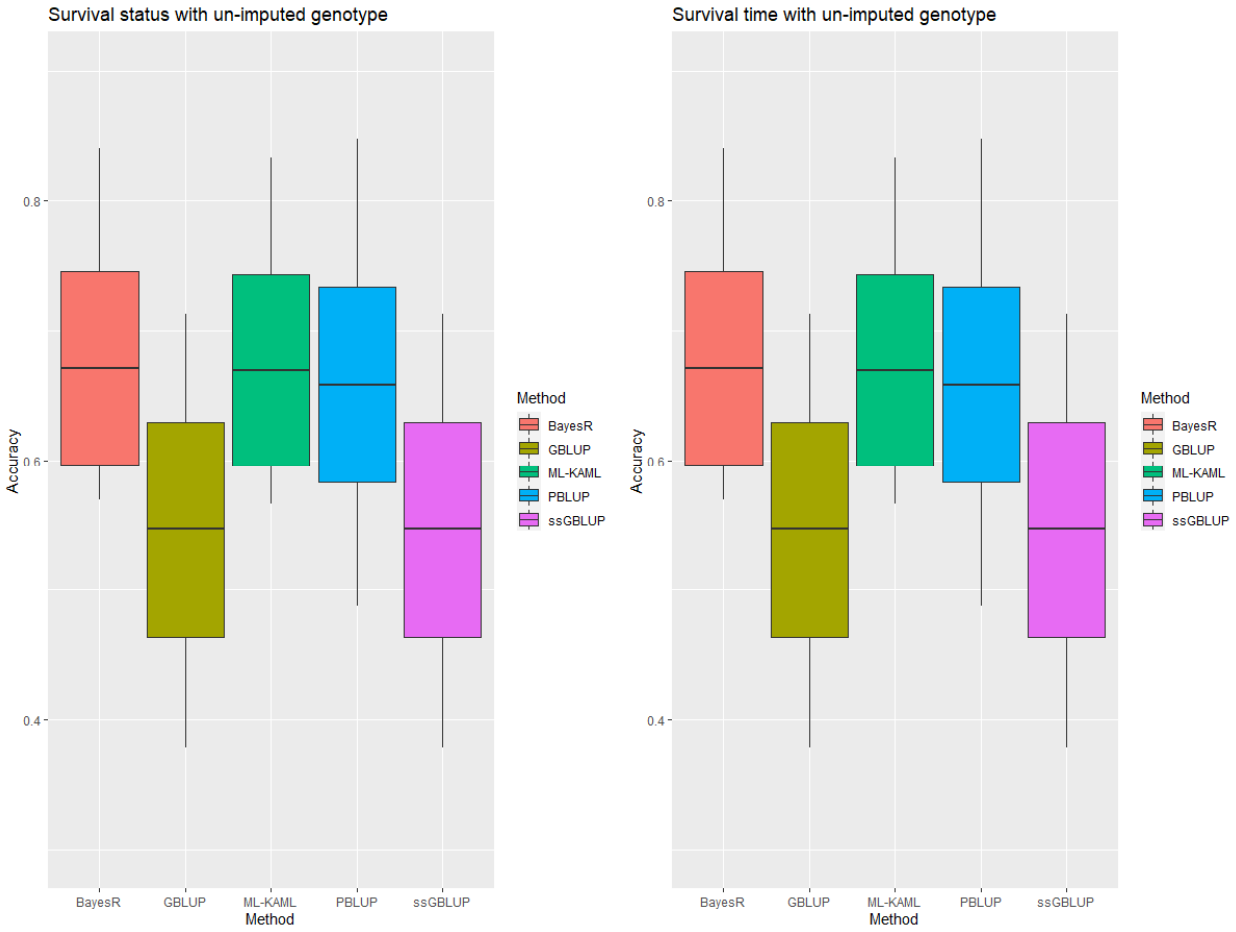


Figure 2. Accuracy of survival status and survival time traits using original genotype. Middle line of the box is mean accuracy; top and bottom lines of the box is accuracy ± one standard deviation. End points of vertical line represent min and max values. Note that PBLUP uses phenotype and pedigree information only.

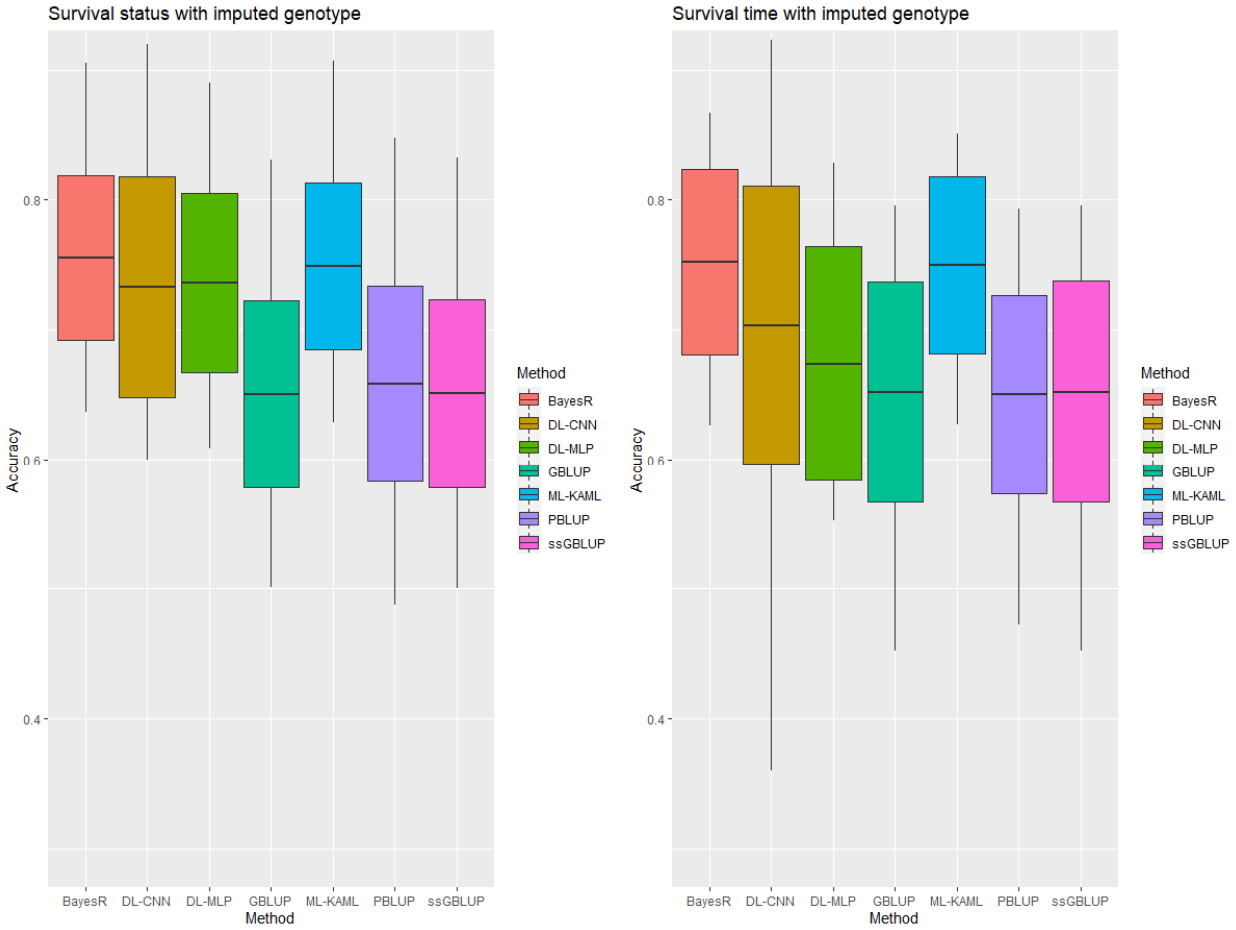


Figure 3. Accuracy of survival status and survival time traits using imputed genotype. Middle line of the box is mean accuracy; top and bottom lines of the box is accuracy ± one standard deviation. End points of vertical line represent min and max values. Note that PBLUP uses phenotype and pedigree information only.

711 Table 1. Number of animals (n) and phenotypes in the disease resistant and susceptible groups used for
712 genotyping.

Groups	20 Lowest survival family		20 Highest survival families		All 40 families	
Trait	Individuals	Mean ± SD	Ind/family	Mean ± SD	n	Mean ± SD
Survival status (%)	245	4.5 ± 20.8	245	60.0 ± 49.1	490	32.2 ± 46.8
Survival time (hour)	245	120.0 ± 52.8	245	207.8 ± 103.9	490	163.9 ± 93.4

713

714

715 Table 2. Variance components and heritability for survival status and survival time using AIREMLf90 and
716 THRGIBBS1f90 sub-program in BLUPF90 and BayesR

Trait	Models	Additive genetic variance	Phenotypic variance	Heritability
Survival status	PBLUP	0.147	0.207	0.71 ± 0.14
	GBLUP	0.086	0.188	0.46 ± 0.09
	ssGBLUP	0.088	0.190	0.46 ± 0.09
	BayesR	0.043	0.044	0.96 ± 0.03
Survival time	PBLUP	9.579	14.694	0.65 ± 0.14
	GBLUP	5.900	13.303	0.44 ± 0.09
	ssGBLUP	5.908	13.442	0.44 ± 0.09
	BayesR	2.747	2.818	0.97 ± 0.02

717 Note that estimate of genetic variance was not provided by ML-KAML, DL-MLP and DL-CNN methods.

718

719 Table 3. Genomic prediction accuracy for survival status and survival time using univariate and bivariate
720 models with un-imputed 6470 SNPs

Trait	Method	Univariate			Bivariate			Increasement (%)
		Accuracy±sd	RMSE	R^2	Accuracy±sd	RMSE	R^2	
Survival status	PBLUP	0.66 ± 0.08	0.39	0.30	0.66 ± 0.07	0.39	0.30	- 0.3
	GBLUP	0.55 ± 0.08	0.41	0.21	0.53 ± 0.08	0.42	0.20	- 2.5
	ssGBLUP	0.55 ± 0.08	0.41	0.21	0.54 ± 0.08	0.41	0.21	- 0.7
Survival time	PBLUP	0.65 ± 0.08	3.30	0.26	0.63 ± 0.09	3.33	0.25	- 2.9
	GBLUP	0.59 ± 0.09	3.41	0.22	0.57 ± 0.07	3.47	0.21	- 3.5
	ssGBLUP	0.61 ± 0.07	3.38	0.23	0.58 ± 0.07	3.47	0.21	- 5.6

721 Note: bivariate analysis is not available in BayesR, ML-KAML, DI-MLP and DL-CNN. RMSE = Root Square
722 Mean Error, R^2 = coefficient of determination, sd = standard deviation. Increasement (%) = (accuracy of
723 bivariate – accuracy of univariate)/accuracy of univariate.

724

725 Table 4. Accuracy of genomic prediction using imputed genotypes of 6470 vs ssGWAS SNPs.

Trait	Method	6,470 SNPs			ssGWAS SNPs			Increasment (%)
Survival status		Accuracy±sd	RMSE	R^2	Accuracy±sd	RMSE	R^2	
	GBLUP	0.65 ± 0.07	0.39	0.29	0.62 ± 0.07	0.40	0.26	- 4.9
	ssGBLUP	0.65 ± 0.07	0.39	0.29	0.62 ± 0.07	0.40	0.27	- 4.3
	BayesR	0.76 ± 0.06	0.36	0.39	0.71 ± 0.06	0.38	0.34	- 6.0
	ML-KAML	0.75 ± 0.06	0.36	0.39	0.72 ± 0.06	0.37	0.36	- 4.0
	DL-MLP	0.74 ± 0.08	0.37	0.38	0.62 ± 0.08	0.40	0.27	- 15.6
	DL-CNN	0.73 ± 0.09	0.37	0.37	0.65 ± 0.07	0.46	0.35	- 11.2
Survival time	GBLUP	0.65 ± 0.09	3.31	0.26	0.64 ± 12.5	3.31	0.25	- 1.8
	ssGBLUP	0.65 ± 0.09	3.31	0.26	0.65 ± 11.4	3.30	0.26	- 0.8
	BayesR	0.75 ± 0.07	3.11	0.35	0.75 ± 0.07	3.11	0.35	- 0.3
	ML-KAML	0.75 ± 0.07	3.10	0.35	0.75 ± 0.07	3.10	0.35	- 0.3
	DL-MLP	0.67 ± 0.09	3.25	0.29	0.64 ± 0.09	3.31	0.26	- 4.9
	DL-CNN	0.70 ± 0.11	3.20	0.30	0.69 ± 0.07	3.25	0.30	- 2.1

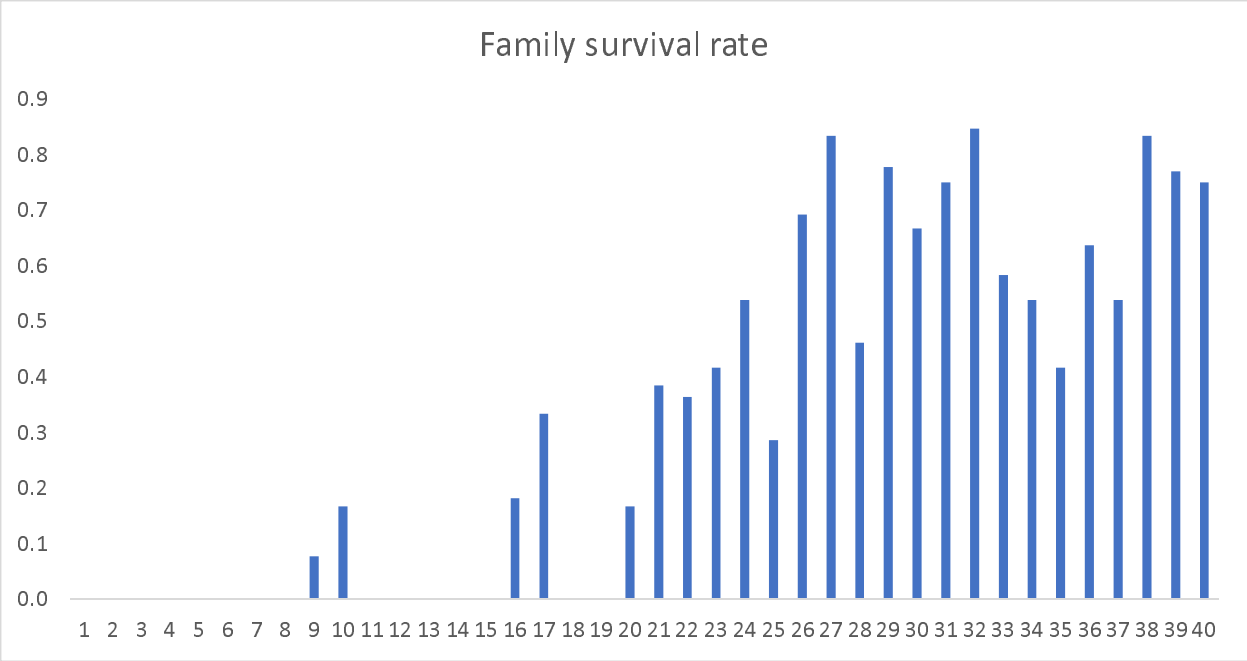
726 6470 SNPs were filtered with dartR packages. ssGWAS SPs were selected based on p-value of each SNPs
727 effect (<0.00001) in each testing set. RMSE = root mean squared error and coefficient of determination,
728 sd = standard deviation. Increasement (%) = (accuracy of ssGWAS SNPs – accuracy of 6470 SNPs)/
729 accuracy of 6470 SNPs

730

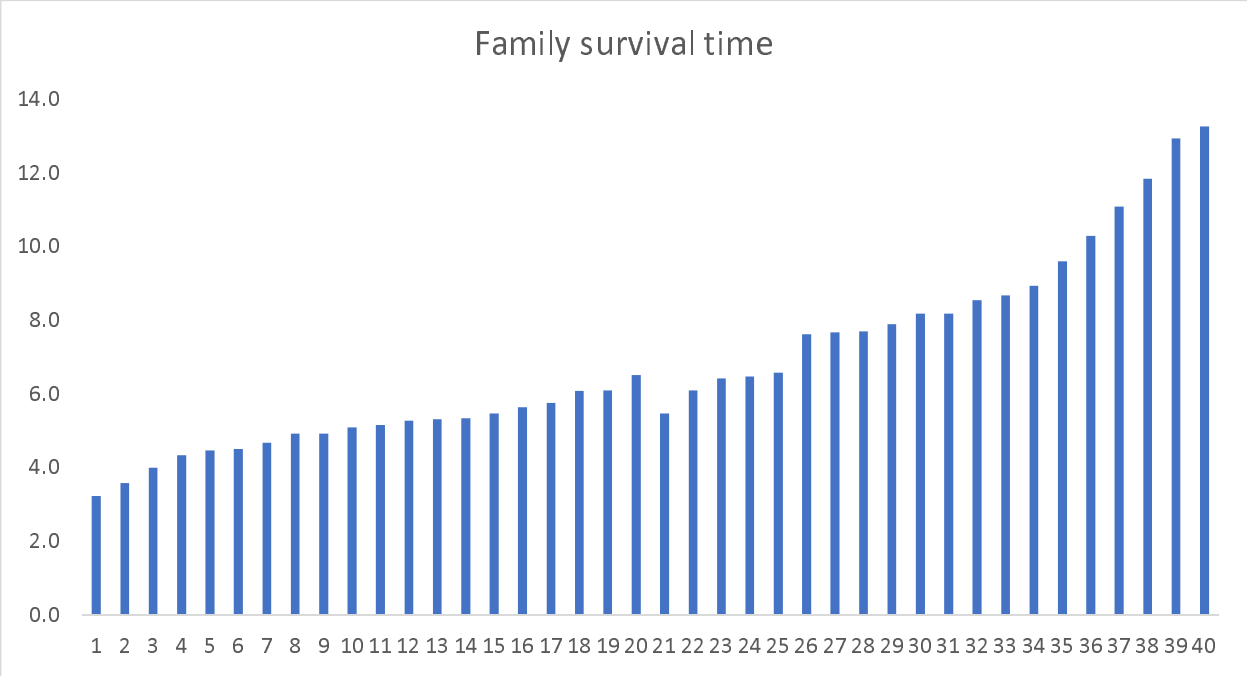
731 Table 5. Accuracy (±sd) of genomic prediction for survival status and survival using highly significant
732 SNPs (P < 0.0001)

Trait	Method	Original (un-imputed) genotype			Imputed genotype		
Survival status		6470 SNPs	ssGWAS SNPs	Increase mnt (%)	6470 SNPs	ssGWAS SNPs	Increase mnt (%)
	PBLUP	0.66	-	-	-	-	-
	GBLUP	0.55	0.48 ± 0.09	- 12.5	0.65	0.62 ± 0.07	- 4.9
	ssGBLUP	0.5	0.49 ± 0.09	- 9.9	0.65	0.62 ± 0.07	- 4.3
	BayesR	0.67	0.62 ± 0.09	- 8.2	0.76	0.71 ± 0.06	- 6.0
	ML-KAML	0.67	0.61 ± 0.09	- 8.5	0.75	0.72 ± 0.06	- 4.3
	DL-MLP	-	-	-	0.74	0.62 ± 0.08	- 15.6
	DL-CNN	-	-	-	0.73	0.65 ± 0.07	- 11.2
Survival time	PBLUP	0.65	-	-	-	-	-
	GBLUP	0.59	0.59 ± 0.12	+ 0.2	0.65	0.64 ± 0.13	- 1.8
	ssGBLUP	0.61	0.60 ± 0.12	- 0.3	0.65	0.65 ± 0.11	- 0.7
	BayesR	0.71	0.70 ± 0.07	- 2.1	0.75	0.75 ± 0.07	- 0.4
	ML-KAML	0.69	0.70 ± 0.06	+ 0.3	0.75	0.75 ± 0.07	- 0.3
	DL-MLP	-	-	-	0.67	0.64 ± 0.09	- 4.9
	DL-CNN	-	-	-	0.70	0.69 ± 0.07	- 2.1

733 Sd = standard deviation; increasement (%) = (accuracy of ssGWAS SNPs – accuracy of 6470 SNPs)/
734 accuracy of 6470 SNPs using un-imputed or imputed genotypes.



Supplementary Figure S1. Mean family survival rate in percentage post challenge test. First 20 family is low resistance group and last 20 families is high resistance group.



Supplementary Figure S2. Mean family survival time in day post challenge test. First 20 family is low resistance groups, and last 20 families is high resistance group.

743 **Supplementary Table S1. Accuracy between methods using non-imputed or imputed genotypes using**
744 **6470 SNPs**

Trait	Method	Non-imputed		Imputed		Increasement (%)
		Accuracy±sd (Pearson)	Accuracy±sd (Mathew's)	Accuracy±sd (Pearson)	Accuracy±sd (Mathew's)	
Binary survival	PBLUP	0.66±0.08	0.47±0.11	-		-
	GBLUP	0.55±0.08	0.39±0.12	0.65±0.07	0.51±0.08	+ 19.0
	ssGBLUP	0.55±0.08	0.39±0.12	0.65±0.07	0.51±0.07	+ 19.2
	BayesR	0.67±0.08	0.55±0.09	0.76±0.06	0.63±0.09	+ 12.5
	ML-KAML	0.67±0.07	0.57±0.10	0.75±0.06	0.63±0.10	+ 12.0
	DL-MLP*	n.e.	n.e.	0.71±0.11	0.65±0.11	
	DL-CNN	n.e.	n.e.	0.73±0.08	0.63±0.12	
Survival time	PBLUP	0.65±0.08	n.e.	-	-	-
	GBLUP	0.59±0.09	n.e.	0.65±0.09	n.e.	+ 10.0
	ssGBLUP	0.61±0.07	n.e.	0.65±0.09	n.e.	+ 7.1
	BayesR	0.70±0.07	n.e.	0.75±0.07	n.e.	+ 5.3
	ML-KAML	0.69±0.07	n.e.	0.75±0.07	n.e.	+ 8.1
	DL-MLP	n.e.	n.e.	0.67±0.09	n.e.	
	DL-CNN	n.e.	n.e.	0.70±0.11	n.e.	

745 n.e. not estimable due to DL-MLP and DL-CNN do not accept un-imputed missing genotype or not
746 estimable for Mathew's coefficient of continuous trait (i.e., survival time). Increasement (%) = (accuracy
747 of imputed 6470 SNPs – accuracy of un-imputed 6470 SNPs)/ accuracy of un-imputed 6470 SNPs

748

749

750 Supplementary Table S2. Number of SNPs used for each testing set based on its effect with p<0.00001

Subset	Total SNP used for ssGWAS	ssGWAS SNPs for survival status	ssGWAS SNPs for survival time
i1t1	6470	342	1448
i1t2	6470	381	1537
i1t3	6470	356	1399
i1t4	6470	351	1561
i1t5	6470	318	1499
i2t1	6470	335	1450
i2t2	6470	366	1598
i2t3	6470	400	1540
i2t4	6470	318	1454
i2t5	6470	370	1401
i3t1	6470	341	1401
i3t2	6470	365	1475
i3t3	6470	373	1514
i3t4	6470	369	1559
i3t5	6470	361	1538
i4t1	6470	372	1576
i4t2	6470	326	1362
i4t3	6470	357	1539
i4t4	6470	349	1383
i4t5	6470	327	1548
i5t1	6470	329	1423
i5t2	6470	367	1480
i5t3	6470	341	1496
i5t4	6470	362	1555
i5t5	6470	360	1502
Mean		353.4	1489.5
Min		318	1362
Max		400	1589

751

752