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## The relation of body score (body height/body length) and haplotype E on Phu Quoc Ridgeback dogs (*Canis familiaris*)

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#### Abstract

Phu Quoc Ridgeback dogs (*Canis familiaris*) were one of the endemic species of Phu Quoc Island – Vietnam, This animal is one of the rarest dog in the world, which has a ridgeback. The haplotype E, one of the breed dogs rare haplotype, high percentage 16.67% in the Phu Quoc dogs observed. By the bootstrap method, we estimated and accurated the morphology characteristics difference between haplotype E (hapE) and others group (nonE) of Phu Quoc ridgeback dogs. The difference of body score (height/length) between haplotype E dogs group and non-haplotype E dogs groups which calculated by bootstrap method [0.001 - 0.068, 95% CI], it is more accurate than t-test methods [-0.007 - 0.077, 95% CI], The results by regression logistic showed that the haplotype E occurrence probability can be predicted based on body score (body height/body length ratio), when this ratio increase 0.046 standard deviation, the haplotype E occurrence probability increase 47%

**Keywords:** Phu Quoc ridgeback dogs, haplotype, morphology, bootstrap, logistic regression

#### 1. Introduction

Phu Quoc ridgeback dogs primarily live in the Phu Quoc island in Vietnam's southern Kien Giang province, Vietnam. They are considered as one of the Vietnamese valuables dogs breeds carrying many good characteristics such as intelligence, strength, good parenting, people friendly and more importantly, still keeping wild characteristics of hunting dogs. Besides Rhodesian ridgeback dog and Thai ridgeback dog, Phu Quoc ridgeback dog is characterized by the ridge of hair running along their back in the opposite direction from the rest of the coat. However, the origin of Phu Quoc ridgeback dogs is undocumented and unclear <sup>[1]</sup> Phu Quoc dog was one of the breed dog in rarest dog group (three breed identified until now), the others were Thai Ridgeback dog and Rhodesian Ridgeback dog. However, at present, only two ridgeback breed dogs were Rhodesian Ridgebacks and Thai Ridgebacks were recognized by Federation Cynologique International (FCI), while Phu Quoc ridge back dog was closely bunched and considered have originated from the Thai Ridgebacks.

Mitochondrial DNA (mtDNA) is the best source of valuable information about the evolutionary history of the domesticated dog <sup>[2, 3]</sup> as well as the origin of some dog breeds <sup>[4]</sup>. mtDNA genome of a domestic dog (*Canis lupus familiaris*) is about 16,727 bp long with the control region (CR) spanning positions 15,458 – 16,727. This 1,270 bp non-coding DNA segment contains two hypervariable regions, HV1 and HV2 <sup>[5, 6]</sup>. Analyzing the phylogenetic relationship of 582 bp sequences in the HV1 of 1,576 dogs, Pang *et al.* (2009a) have found that all sequences were grouped into six previously described distinct haplogroups A, B, C, D, E, F. In which, 71.3% of sequences were found in haplogroup A; 17.26% in haplogroup B, 7.80% in haplogroup C, 1.78% in haplogroup D, 0.63% in haplogroup E and 0.19% in haplogroup F <sup>[2]</sup>.

Thai Ke Quan *et al.* (2016) analyzing 582 bp mtDNA HV1 sequences of 30 Phu Quoc ridgeback dog living in Phu Quoc island, 28 SNPs and 11 haplotypes belonged to 4 haplogroups A, B, C and E were identified. It showed the high genetic diversity of the population. The amazing results is the high frequency of Phu Quoc ridgeback dogs harbouring haplogroup E (16.67%) which are rare distribution all over the world <sup>[7]</sup>

Based on the results from the previous research in Vietnam, we study to understand the difference between the haplotype E and others haplotype based on morphology parameters, include body length, body height, waist and chest. Addition, we estimated the occurrence

probability haplotype E based on ratio body height /body length by logistic regression in R programe.

## 2. Materials and Methods

### 2.1 Phenotypic parameters measurement

Total includes 30 Phu Quoc Ridgeback dogs were observed and recorded for the basic measurements to quantify include body height, body length, chest and waist (measurement according to the principles prescribed by the Fédération Cynologique Internationale). All dogs observed in the mature phase and could reproduce <sup>[8]</sup>

### 2.2 Phenotypic parameters analysis by bootstrapping method

In statistics, bootstrapping can refer to any test or metric that relies on random sampling with replacement. Bootstrapping allows assigning measures of accuracy (defined in terms of bias, variance, confidence intervals, prediction error or some other such measure) to sample estimates.

The bootstrap was published by Bradley Efron. It was inspired by earlier work on the jackknife. Improved estimates of the variance were developed later <sup>[9]</sup>.

The basic idea of bootstrapping is that inference about a population from sample data (sample → population) can be modelled by re sampling the sample data and performing inference on (re sample → sample). As the population is unknown, the true error in a sample statistic against its population value is unknowable. In bootstrap-resamples, the 'population' is in fact the sample, and this is known; hence the quality of inference from re sample data → 'true' sample is measurable. Bootstrap method widely used in studies on animals behaviour, the interaction between phenotype and phenotype in breeding cattle selection <sup>[10-12]</sup>

In this research, the 30 Phu Quoc ridgeback dogs were bootstrapped 1000 times by simple R code. Data collected from bootstrapping method compared with data from original t-test to optimal the difference between halotype E group with the others based on phenotype basic characteristics.

R code used to bootstrap phenotype and haplotype data collected in 30 Phu Quoc ridgeback dogs (below):

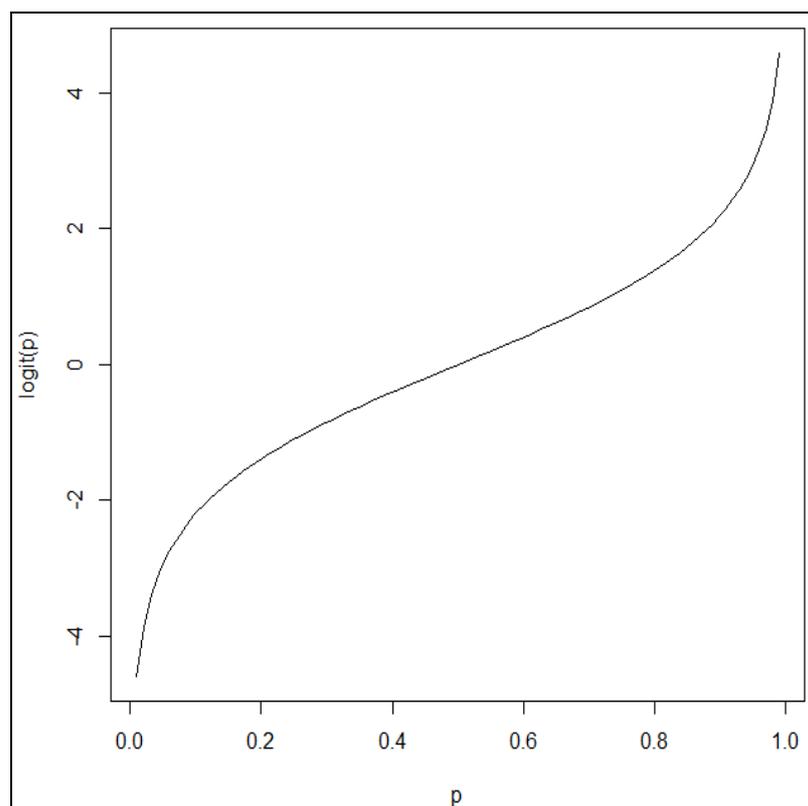
```
nonE = c(data) (phenotype data of non-haplotype E dogs collected )
hapE = c(data) (phenotype data of haplotype E dogs collected )
n1 = length(nonE) (non-haplotype E numeric data identified)
n2 = length(hapE) (haplotype E numeric data identified)
B = 1000 (bootstrap 1000 times)
difference = numeric(B) (setting up difference parameter)
for (i in 1:B){
  bs.nonE <- sample(nonE, n1, replace=T)
  bs.hapE <- sample(hapE, n2, replace=T)
  difference[i] = mean(bs.hapE) - mean(bs.nonE)
} (re-sampling and quantification difference parameter)
```

### 2.3 Logistic regression model using evaluate the haplotype E occurrence probability based on phenotypic characteristics of Phu Quoc ridgeback dogs

Logistic regression, or logit regression, or logit model <sup>[13]</sup> is a regression model where the dependent variable is categorical, where it can take only two values, such as pass/fail, win/lose, alive/dead or healthy/sick. Cases with more than two categories are referred to as multinomial logistic regression, or, if the multiple categories are ordered, as ordinal logistic regression <sup>[14]</sup>

Logistic regression was developed by statistician David Cox in 1958. The binary logistic model is used to estimate the probability of a binary response based on one or more predictor (or independent) variables (features) <sup>[14]</sup>. As such it is not a classification method. It could be called a qualitative response/discrete choice model <sup>[15]</sup>.

In this research and investigation, the Phu Quoc ridgeback dogs phenotype parameters were collected and dissected. Based on analysis values of phenotype and genotype, we evaluate occurrence probability of haplotype E compared to other haplotypes in Phu Quoc ridgeback dogs population (haplotype E occur: yes/no).



**Fig 1:** The correlation of  $\text{logit}(p)$  and  $p$ , given  $1 > p > 0$ .

Observed for a frequency  $m$  (Phu Quoc ridgeback dogs with haplotype E) recorded events from  $n$  objects sampling (30 Phu Quoc ridgeback dogs), the probability of the event occurring is  $p$ :

$$p = \frac{m}{n}$$

and the ability of this event is *odds*, this is the ratio of probability of the event and the probability of the non-event:

$$odds = \frac{p}{(1-p)}$$

logit function of *odds* is determined:

$$\text{logit}(p) = \log \frac{p}{(1-p)}$$

The relationship between  $p$  and  $\text{logit}(p)$  is a continuous relationship (Figure 1). If we collect the morphology characteristic data which called  $x$  (independent or dependent variable) and logistic regression models represented the relationship between  $\text{logit}(p)$  and  $x$  given by function:

$$\text{logit}(p) = \alpha + \beta x \quad (*)$$

In this study we wanted to know the relationship between the ratio of body height and body length (BH/BL) with the ability to appear haplotype E on Phu Quoc ridgeback dogs population. If called BH/BL is  $x$ , the problem is represented as a function:

$$\begin{aligned} \text{logit}(p) &= \log \frac{p}{(1-p)} = \alpha + \beta x \\ \text{or } odds(p) &= \frac{p}{(1-p)} = e^{\alpha + \beta x} \end{aligned}$$

Regression model states that the relationship between the probability of occurrence haplotype E and BH/BL is an S relationship-shaped. The model also shows the probability of haplotype E  $p$  depends on the value of  $x$ . The model can more accurately presents that the possible occurrence of haplotype E ( $p$  or odds) with conditions  $x$  (BH/BL):

$$odds = (p|x) = e^{\alpha + \beta x}$$

When  $x = x_0$ , the probability occurrence haplotype E:

$$odds(p|x = x_0) = e^{\alpha + \beta x_0}$$

When  $x = x_0 + 1$ , the probability occurrence haplotype E:

$$odds(p|x = x_0 + 1) = e^{\alpha + \beta(x_0 + 1)}$$

and the *odds ratio* between  $x = x_0$  and  $x = x_0 + 1$ :

$$\frac{odds(p|x = x_0 + 1)}{odds(p|x = x_0)} = \frac{e^{\alpha + \beta(x_0 + 1)}}{e^{\alpha + \beta x_0}} = e^{\beta}$$

The  $e^{\beta}$  was call *odds ratio* (OR). To estimate the  $\alpha$  and  $\beta$ , the methods used are weighted iterative least square or Newton-Raphson. R programe used Newton-Raphson method to find  $\alpha$  and  $\beta$ :

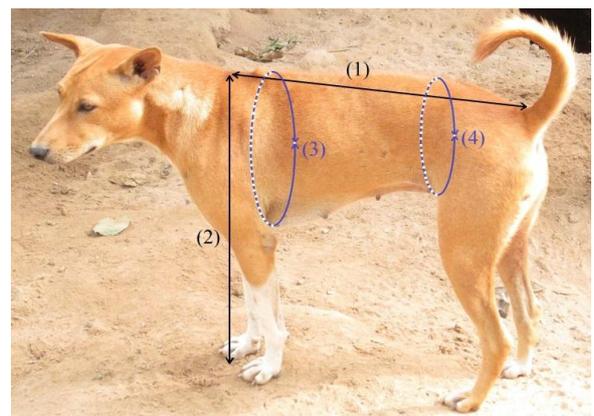
$$p = \frac{e^{\alpha + \beta x}}{1 + e^{\alpha + \beta x}} = \frac{1}{1 + e^{-(\alpha + \beta x)}}$$

Once the  $\alpha$  and  $\beta$  have estimated, can calculate the probability  $p$  for any  $x$  value

### 3. Results and Discussion

#### 3.1 Phenotypic parameters measurement

All dogs observed in the mature phase and could reproduce. Sampling location and experiments measure is Thanh Nga Kennels, Phu Quoc Island, Vietnam from November 2014 to January 2016. Total includes 30 Phu Quoc Ridgeback dogs were observed and recorded for the basic measurements which include chest, waist, body height and body length (measurement according to the principles prescribed by the Fédération Cynologique Internationale).



**Fig 2:** Phenotypic parameters of Phu Quoc ridgeback dogs. (1) body length, (2) body height, (3) chest, (4) waist

The body height (BH), body length (BL), Chest (CH), Waist (WA) were measured in centimeters (cm); Ratio height/length (BH/BL) and Chest/ Waist were calculated simply. The phenotypic indicators assess the relevance to occurrence possible of haplotype E compared to another haplotype in Phu Quoc ridgeback dogs. Haplotype E was interested in by dogs breeder because it is very rare haplotype on accient dogs over the world. Phu Quoc ridgeback dogs living in Vietnam carry this haplotype which present on high frequency (16.67%) [7]

**Table 1:** The basic phenotype parameter and haplotype of 30 Phuc Quoc ridgeback dogs. Haplotype data determined by Thai ke Quan *et al.* (2016), was permitted by author [7]

Number	BL	BH	BH/BL	Chest	Waist	CH/WA	Haplotype E
PQ33	47	45	0.957	56	48	1.167	0
PQ34	48	44	0.917	48	41	1.170	0
PQ35	50	45	0.900	50	42	1.190	0
PQ36	50	48	0.960	57	49	1.163	0
PQ37	51	50	0.980	60	54	1.111	0
PQ38	53	45	0.849	54	46	1.174	0

PQ39	52	45	0.865	43	38	1.132	0
PQ40	57	50	0.877	60	45	1.333	0
PQ41	50	46	0.920	61	51	1.196	0
PQ42	52	47	0.904	52	45	1.156	0
PQ43	48	45	0.938	57	47	1.213	0
PQ44	57	52	0.912	70	57	1.228	0
PQ45	57	51	0.895	57	44	1.295	0
PQ46	50	48	0.960	60	48	1.250	0
PQ47	52	47	0.904	58	46	1.260	0
PQ51	58	57	0.983	60	52	1.154	0
PQ52	62	55	0.887	64	49	1.306	0
PQ53	50	40	0.800	46	37	1.243	0
PQ54	53	50	0.943	58	48	1.208	0
PQ55	46	43	0.935	60	50	1.200	0
PQ57	48	45	0.938	50	40	1.250	0
PQ58	55	48	0.873	53	42	1.262	0
PQ59	53	49	0.925	58	51	1.137	0
PQ60	54	52	0.963	58	50	1.160	0
PQ62	45	37	0.822	50	39	1.282	0
PQ48	50	47	0.940	54	43	1.256	1
PQ49	50	49	0.980	59	49	1.204	1
PQ50	60	55	0.917	63	52	1.212	1
PQ56	53	52	0.981	52	43	1.209	1
PQ61	55	50	0.909	60	50	1.200	1

**3.2 Phenotype characteristics analysis and compare between Haplotype E and others haplotype group of Phu Quoc ridgeback dogs by bootstrap method**

This investigation based on 30 Phu Quoc ridgeback dogs which collected phenotype characteristics data, include 5 in haplotype E group and 25 in others haplotype group. Using the bootstrap method, we need to understand if this investigation repeats 1000 times, and take 30 dogs per time, the ratio BH/BL and CH/WA which evaluated and compared with original t-test based on 30 dogs will how changed and

transformed. Assessment by t-test method of the differences between both two dog groups in BH/BL and CH/WA have no signification when the coefficient p-value > 0.05 (BH/BL: 0.103 and CH/WA: 0.345), but p-value (0.103) show the relative difference can acceptable, that mean the difference in BH/BL between haplotype E groups and others haplotype group is low significance. The p-value (0.345) show that the difference in CH/WA between haplotype E groups and others haplotype group is no significance.

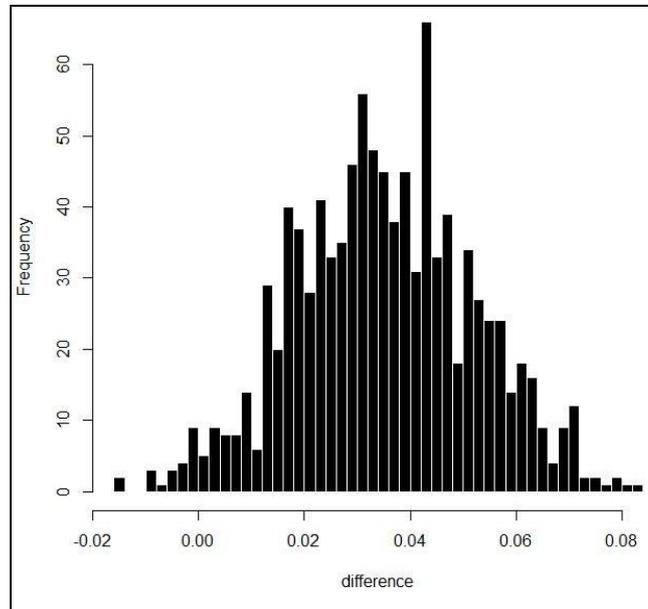
**Table 2:** The data analysis results of Phu Quoc ridgeback dogs phenotype characteristics

Phenotype characteristics		Bootstrap (1000 times)		T-test	
		HapE	NoneE	HapE	NoneE
BH/BL	Mean	0.915	0.907	0.945	0.912
	Median	0.909	0.916	0.940	0.915
	95% CI	[0.001 - 0.068]		[-0.007 - 0.077]	
	<i>p-value</i>	<i>difference average: 0.035</i>		<i>p-value = 0.103</i>	
CH/WA	Mean	1.216	1.187	1.216	1.202
	Median	1.209	1.196	1.209	1.196
	95% CI	[-0.012 - 0.043]		[-0.017 - 0.046]	
	<i>p-value</i>	<i>difference average: 0.014</i>		<i>p-value = 0.345</i>	

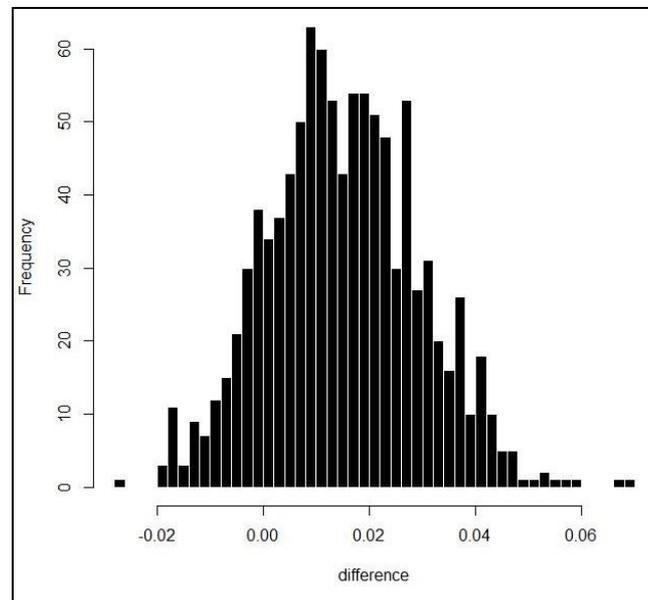
**HapE:** Haplotype E group; **NoneE:** Others haplotype group; **BH/BL:** Body Height/Body Length; **CH/WA:** Chest/Waist; CI: confidence interval

When using the bootstrap method, the data were optimized and increased accuracy transformed to the normal distribution data. A great advantage of bootstrap is its simplicity. It is a straightforward way to derive estimates of standard errors and confidence intervals for complex estimators of complex parameters of the distribution, such as percentile points, proportions, odds ratio, and correlation coefficients (figure 2). The average difference is 0.035 and the 95% CI of variability ratio BH/BL between two group dogs from 0.001 to 0.068, this mean the average ratio BH/BL value of haplotype E group higher 0.034 than others haplotype group and 95%

individuals of haplotype E higher from 0.001 to 0.068 than others haplotype in Phu Quoc ridgeback dogs population. This difference has low significance (p-value = 0.103). The 95% CI of bootstrap smaller than CI of t-test so that the results with bootstrap making it a more precise estimate of the Phu Quoc ridgeback dogs population [15]. Similarity, the ratio CH/WA of bootstrap group show the 95% confidence interval smaller than t-test group, But the ratio had p-value so high (0.345), that mean there is no difference between haplotype E and others haplotype group based on this ratio of these phenotype characteristics (chest/waist).



**Fig 3a:** BH/BL histogram of difference between haplotype E group and others group of Phu Quoc ridgeback dogs with bootstrap 1000 times.



**Fig 3b:** CH/WA histogram of difference between haplotype E group and others group of Phu Quoc ridgeback dogs with bootstrap 1000 times

**3.3 Relationship between body height/body length (BH/BL) and haplotype E using logistic regression model of Phu Quoc ridgeback dogs sampling**

The results shows that the BH/BL in haplotype E group higher than others group (0.945 >0.910), p-value (0.103)

show the relative difference can acceptable, that mean the difference in BH/BL between haplotype E groups and others haplotype group is low significance. (Table 2)

The generalized linear model function (glm) in R program based on Newton-Raphson method to find estimated  $\alpha$  and  $\beta$ .

**Table 3:** Results of *glm* function to estimate the  $\alpha$  and  $\beta$  in (\*) equation

Function	glm(formula = fx ~ BH/BL, family = "binomial")				
	Min	1Q	Median	3Q	Max
Deviance Residuals:	-0.9784	-0.6491	-0.4634	-0.2741	2.0925
Coefficients:	Estimate Std. Error z-value Pr(> z )				
	(Intercept)	-21.51	14.09	-1.527	0.127
	BH/BL	21.38	14.96	1.429	0.153
Standard deviation (BH/BL)	0.046				

The function glm used to understand the relationship between the ration BH/BL (x) and haplotype E occurrence probability (fx). Because the fx distribution is binomial (yes or no), the binomial is the standard distribution for logistic regression model in this research.

Deviance shows the difference of predicted value and the observed value of logit (p). It's similar mean square residual

in linear regression model. In this research, the deviance is lightly significance. In the coefficients statistic, intercept and BH/BL which used to estimate the  $\alpha$  and  $\beta$  parameter, with estimated intercept =  $\alpha$  = -21.51 and estimated ratio BH/BL =  $\beta$  = 21.58. The estimated  $\beta$  shows the relationship between ratio BH/BL and the haplotype E occurrence probability is positive, this means that when this ratio increases, the

haplotype E occurrence probability of increases. Addition, z-value is the estimated parameter/standard error (21.38/14.96 = 1.429) with p-value = 0.153 shows us the ratio BH/BL does not affect the appearance of haplotype E on Phu Quoc ridgeback dogs, it's just relationship with the haplotype occurrence probability.

Standard deviation is 0.046 and the odds ratio (OR) was calculated based on bias unit 0.046 and the equation to calculated p based on ratio BH/BL:

$$p = \frac{e^{\alpha + \beta x}}{1 + e^{\alpha + \beta x}} = \frac{1}{1 + e^{-(\alpha + \beta x)}}$$

replace the numbers to calculate on equation:

$$p = \frac{1}{1 + e^{-(-21.51 + 21.38 \cdot BH/BL)}}$$

When ratio BH/BL = 1:

$$p = \frac{1}{1 + e^{-(-21.51 + 21.38 \cdot 1)}} = 0.47$$

When ratio BH/BL decrease one unit or one standard deviation (0.046), BH/BL = 1 - 0.046 = 0.954:

$$p = \frac{1}{1 + e^{-(-21.51 + 21.38 \cdot 0.954)}} = 0.25$$

Then, if ratio BH/BL decrease one standard deviation (0.046), the haplotype E occurrence probability of Phu Quoc ridgeback dogs changed 0.25/0.47 = 0.53 or (0.25/0.47-1) \*100% = (-47%). The negative percent number means the occurrence probability decrease 47%.

The data collected had ratio BH/BL min = 0.8 and max = 0.983. The statistic logistic model estimated the probability of haplotype E occurrence on Phu Quoc dogs based on ratio BH/BL. This relationship is positive that mean the occurrence probability decrease when ratio BH/BL decrease and the standard deviation is 0.046 bias unit. Thus, the occurrence probability decreases 47% if ratio BH/BL decrease 0.046 bias unit.

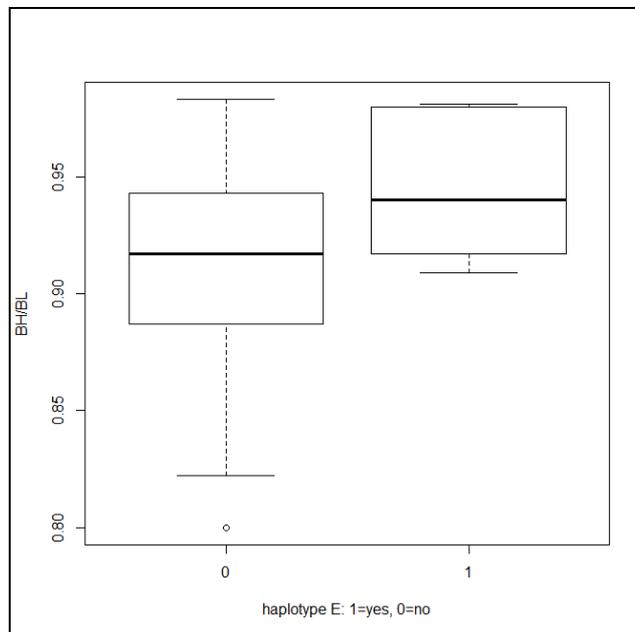


Fig 4: ratio BH/BL in haplotype E group and others haplotype group of Phu Quoc ridgeback dogs observed

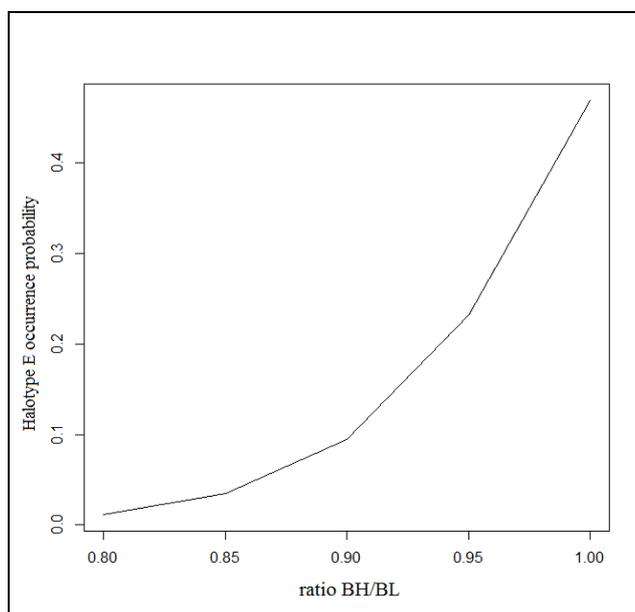


Fig 5: The haplotype E occurrence probability and the ratio BH/BL of Phu Quoc ridgeback dogs observed

#### 4. Conclusion

In the initial study of understanding a process of evaluating the phenotypic characteristics of Phu Quoc Ridgeback dog through Bootstrap methods. The results show that this method increases the accuracy of estimates the characteristics of Phu Quoc dogs phenotype compared with t-test, classical statistical methods. The convenience and versatility of this method are that we can statistics on the small sample size for the population.

Phu Quoc ridgeback dogs breed is endemic species which just living in Phu Quoc Island, Vietnam. Through this original research, the haplotype E ratio is one of the rare haplotype of breeding dogs, representing conserved of the breed dogs in the world has a high rate is 16.67% in Phu Quoc sample size observed.

With logistic regression methods, we can estimate the occurrence probability of haplotype E on Phu Quoc ridgeback dogs based on basic phenotype, the ratio of body height and body length. The results showed that if this ratio decreases a standard deviation (0.046) then the haplotype E occurrence probability decrease 47%.

Addition, the results from this study can be used as a first step testing to predict the likelihood haplotype E type appears on the Phu Quoc dog populations.

#### 5. Acknowledgments

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