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Genetic Variability Analysis of Duck-PRL Exon 5 in Kuttanad Ducks of Environmentally Challenged Breeding Tracts

Bindya Liz Abraham, Priya Melepat

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ABSTRACT

Kuttanad ducks are native ducks reared for eggs and meat in Alappuzha, Ernakulam, Kottayam and Pathanamthitta districts of Kerala. The calamities of duck influenza outbreaks and severe floods in these regions had wiped off lakhs of elite duck clusters, deranged the ecosystem of their breeding tract and aggravated the decline of duck germplasm in the State. In this context, the present study was undertaken to analyze the performance of Kuttanad ducks along with their genetic variability status with respect to a major candidate gene, prolactin (duck-PRL) in the flood-hit populations. The study revealed an optimum growth and egg production performance indicating their resilience as a native variety to climatic adversities. PCR amplification, Single Strand Conformation Polymorphism analysis and sequencing of the 204 base pair duck-PRL exon 5 locus of 160 genomic DNA samples revealed the gene fragment to be monomorphic and highly conserved suggesting a low or negligible genetic variation in the flood-hit duck clusters. The considerable loss of genetic variability at the duck-PRL exon 5 locus is indicative of the severe decline in duck biodiversity that has emerged out of the limited outbreeding of the ducks under severe loss and fragmentation of the breeding tract following the devastating floods.

Keywords Prolactin gene, Exon 5, Kuttanad ducks.

INTRODUCTION

The local ducks found in Kerala popularly known as Kuttanad ducks are appreciated for their dual purpose utility and are reared by around 4000 farmers in the breeding tracts of Alappuzha, Ernakulam, Kottayam and Pathanamthitta Districts of the State. The low-lying lands of these districts constitute Kuttanad region that lies below the Mean Sea Level (MSL) and is characterized by backwaters and paddy fields, globally known for the practice of below-sea level paddy farming. The ducks of Kuttanad share a symbiotic relationship with the paddy farmers of the region. The harvested rice fields are opened to the ducks to feed on the insects, snails and weeds and thus they play a significant role in pest control. The ducks also lay eggs on these fields and duck meat has emerged as a culinary delight of the tourist menu. The breeding tract of these ducks in Kuttanad was badly hit by avian influenza (H5N1) outbreaks in 2014-15 and 2016-17. Following the Avian Influenza (H5NI) outbreaks, over 10 lakh breeder ducks of Kuttanad were culled and many of their fertile eggs

Bindya Liz Abraham*, Priya Melepat
Center for Advanced Studies in Animal Breeding and Genetics
College of Veterinary and Animal Sciences, Mannuthy, Thrissur -680651, Kerala, India
Email: binliza@gmail.com
*Corresponding author

were destroyed (Paul 2020). The devastating floods of 2018 also fragmented their breeding tract and deranged the ecosystem (Anonymous 2018). Several elite duck clusters and ducklings were also drowned in the floods aggravating the decline of duck germplasm in the State. In this context, an assessment of the growth and production performance of Kuttanad ducks in the post-flood scenario is most essential to assess their resilience as a native variety towards the climate change challenges like floods which caused environmental degradation of their breeding tract. Moreover, a scientific analysis of the genetic variability of important genes controlling growth and egg production traits in the duck populations surviving after the avian influenza outbreaks and floods is also likely to facilitate the formulation of future strategies for their conservation, selection and multiplication to save them from the verge of extinction. Therefore the present study aimed at the performance evaluation and genetic variability analysis of prolactin (PRL) gene in Kuttanad ducks following climatic challenges like floods, environmental degradation and disease outbreaks.

Prolactin (PRL) gene is known as an important candidate gene for growth (Irma *et al.* 2014, Mazurowski *et al*. 2016) egg production and egg quality (Li *et al*. 2009, Cui *et al.* 2011) in ducks. The gene is of 10 kb size consisting of five exons and four introns, encoding 229 amino acids for prolactin, an anterior pituitary hormone regulating the onset of incubation, brooding behavior and follicular development (Chang *et al*. 2012). Hence, the present study was undertaken to assess the performance of Kuttanad ducks in their breeding tract and to detect the genetic variability of exon 5 locus of duck-PRL gene in these *desi* duck populations.

MATERIALS AND METHODS

Sampling and phenotypic data collection

A total of 80 Kuttanad ducks belonging to different farming clusters found during 2017-2018, in the breeding tract hit twice by avian influenza outbreaks and heavy floods were evaluated for their growth and production performance. Body weights at hatch and at 20, 40 weeks of age, age at first egg, body weight at first egg, egg weights at 40 and 52 weeks of age and the annual egg production at 72 weeks of age were recorded. Blood samples of 2 ml each were collected in EDTA vacutainers from wing vein of 160 ducks belonging to four talukas viz., Ambalapuzha, Chengannur, Cherthala and Kuttanad of the breeding tract and stored at-20 ^{o} C for further DNA extraction.

DNA extraction and PCR amplification

Genomic DNA was extracted using standard phenol-chloroform method (Sambrook and Russell, 2001) and frozen at -20^{o} C until use. Purity of DNA was detected by NanoDrop@2000 spectrophotometer. Genomic DNA was used for the amplification of 204 bp fragment (exon 5) of PRL gene using primers designed by the Primer 3 software (V.4.0) http://bioinfo.ut.ee/primer30.4/). The PCR primers for PRL exon 5 were synthesized (Sigma-Aldrich, USA) as follows: PRL (exon *5*) - F: 5′-TTCATTCTGGCGACAGC-3" and PRL (*exon 5*) - R: 5′-GAAGCCCAGGAGTACTTAGCCG-3". The PCR was conducted in a 20µL reaction mixture containing 1µl of DNA, 2 µl of 10 × PCR buffer, 1 µl of 2mM of $MgCl_2$, 0.4µl of dNTPs, 1µl of 10 pmol of each primer and 0.2 µl of *Taq* DNA polymerase. The PCR protocol involved an initial denaturation at 94 ^{o}C for 30s followed by 35 cycles of denaturation at 94 ^{o}C for 15s, annealing at 63 ^{o}C for 20s, extension at 72 ^{o}C for 30 s and final extension at 72 ^{o}C for 5 min (Bio-Rad Thermal cycler, USA). Electrophoresis of PCR products was performed in 2% agarose gel in parallel with 50 bp DNA marker (Fermentas) in 1 × TBE buffer at a constant voltage of 80 V for 45 min. After ethidium-bromide staining, products were visualized by ultraviolet transilluminator (Bio-Rad, USA).

PCR-SSCP analysis

The PCR product was then subjected to single-strand conformation polymorphism (SSCP) analysis using vertical electrophoresis (Hoefer, USA). Aliquots of 10 µL of PCR products were mixed with a 20 µL of denaturing solution (containing 9.5 ml of deionized formamide, 0.4 ml of 0.5M EDTA, 2.5 mg of xylene-cyanole and 2.5 mg bromophenol blue) centrifuged, denatured by heating at 95^{o}C for 10 min and immediately chilled on ice. Denatured amplicons

were loaded on 12% PAGE gel in 1x TBE buffer at a constant voltage of 130 V for 18 h. The gel was stained by silver staining to identify polymorphisms if any, at the locus.

DNA sequencing analysis

The PCR product from the single SSCP pattern obtained were sequenced using a commercial service (Sci Genom Labs Pvt Ltd Cochin) in forward and reverse directions to confirm the variability status at the locus.

Statistical analysis

The phenotypic data on body weights and egg production collected from the breeding tract were averaged and standard errors estimated.

RESULTS AND DISCUSSION

Kuttanad ducks in the study were maintained by farmers in their home tract under free range system of management. The estimates of their various growth and production traits are given in Table 1. The study found that the performance of Kuttanad ducks in the post-calamity scenario with respect to the growth and production traits were comparable with the earlier reports on these populations reared under various agro-climatic zones of the country (Mahanta *et al*. 2009; Anitha *et al.* 2012). This was indicative of the innate resilience and hardiness of the Kuttanad ducks as a local indigenous variety surviving and performing well even in an ecosystem hit by floods and disease outbreaks.

Table 1. Growth and production performance of Kuttanad ducks in the breeding tract. *Figures in parenthesis indicate the number of observations (n).

Traits	Mean ± SE*
Body weight at hatch (g)	46.34 ±1.03 (80)
Body weight at 20 weeks (kg)	1.43 ±1.13 (58)
Age at first egg (days)	139.2 ±2.34 (58)
Body weight at first egg (kg)	1.43 ±1.13 (58)
Body weight at 40 weeks of age (kg)	1.58 ±1.36 (55)
Egg weight at 40 weeks of age (g)	60.6 ±2.33 (55)
Egg weight at 52 weeks of age (g)	67.5 ±2.12 (50)
Body weight at 52 weeks of age (kg)	1.65 ±1.54 (50)
Body weight at 72 weeks of age (kg)	1.99 ±1.63 (40)
Annual egg production at 72 weeks of age (n)	192.60 (40)
Mortality below eight weeks of age (%)	23.30 (60)

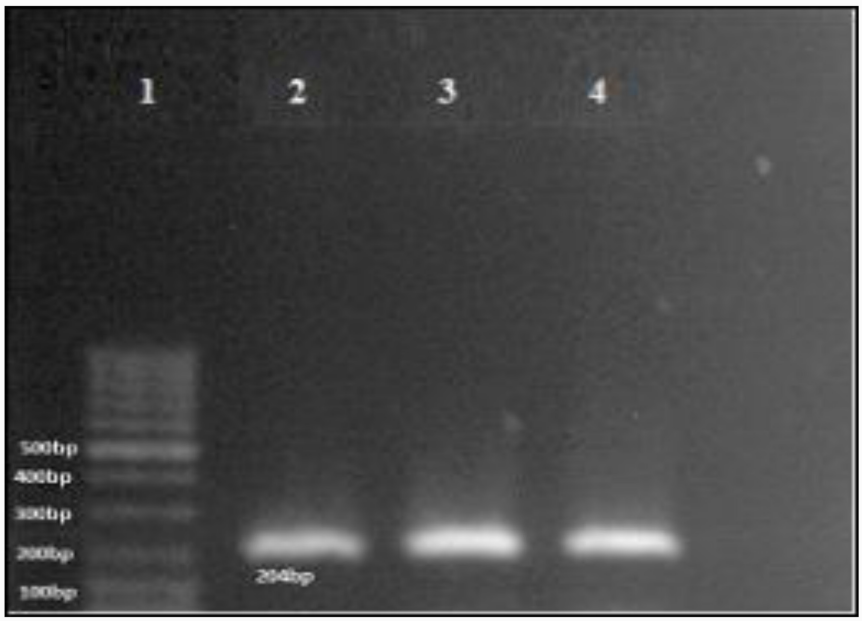

Fig. 1. PCR amplification of 204 bp fragment of duck-PRL gene (exon 5) in Kuttanad ducks.
Lane 1: 100 bp DNA marker,
Lane 2 - 4: 204 bp product.

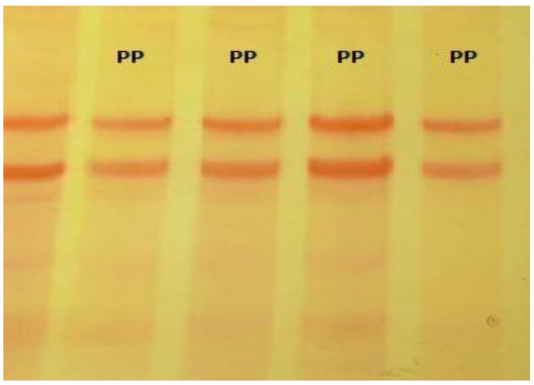

Fig 2. SSCP pattern of 204 bp fragment of duck- PRL gene *(exon* 5) in Kuttanad ducks.

The PCR-SSCP analysis of the exon 5 fragment (204 bp) of duck-PRL gene revealed only one distinct banding pattern in the duck population (Figs. 1 and 2). Sequence analysis further confirmed the duck-PRL gene in Kuttanad ducks to be devoid of any nucleotide polymorphism for the exon 5 locus and hence, only a single genotype (PP) was observed in the population (Fig. 3).

The study has pointed out that being monomorphic, the exon 5 fragment of duck-PRL gene in Kuttanad ducks can be considered as a highly conserved locus with low or negligible genetic variation. The various exonic and intronic loci of duck-PRL gene are generally reported to be highly polymorphic in many duck breeds of Indian and exotic origin (Cui *et al.* 2011, Chang *et al*. 2012, Irma *et al*. 2014, Mazurowski *et al*. 2016). Therefore, the monomorphic status of the exon 5 fragment of duck-PRL in the Kuttanad variety under study is a finding contrary to it's more common polymorphic status in most duck breeds. The reasons commonly attributed towards any loss of genetic variability of important genes governing performance traits in animal populations are either a lowered population size and the subsequent genetic drift operating in the populations (Falconer 1960) or the chances of limited interbreeding of the population on account of some kind of habitat loss or habitat fragmentation of man-made or environmental origin (Wills 1981). In the present context, the repeated disease outbreaks of avian influenza had contributed to a sharp decline in the number of breeding ducks in the region. The devastating floods in the region in consecutive years had also paved way for the loss and fragmentation of the habitat of these native ducks. The present study throws light on the probability that these conditions must have led to a low rate of outbreeding among the surviving duck populations in the breeding tract. Subsequently, there was loss of genetic variability at the important locus of duck-prolactin gene (duck-PRL exon *5*). The finding of the study is indicative of the declining duck biodiversity in the region under climatic challenges which is a matter of concern in the ecological context.

The present study revealed the growth and egg production of Kuttanad ducks in their disaster-hit breeding tract to be conforming to the optimum performance standards and this pointed out the resilience and hardiness of these native duck populations in the post- calamity ecosystem. However, the commonly polymorphic exon 5 locus of duck-PRL gene was found to be monomorphic with only a single genotype in Kuttanad ducks. This finding of the study

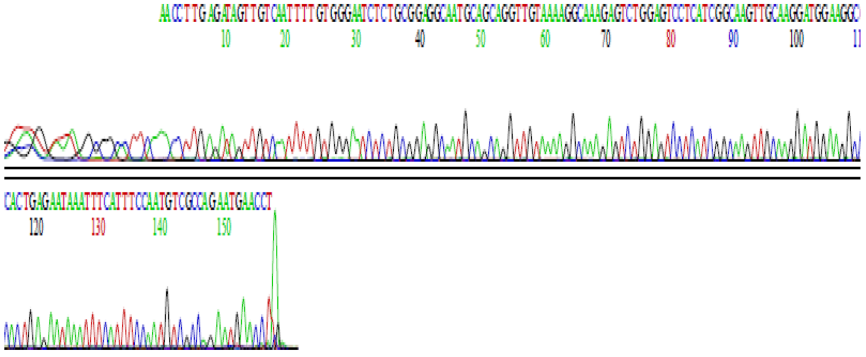

Fig. 3. Sequence map of PP genotype of duck-PRL gene (exon 5) in Kuttanad ducks.

indicated that there was considerable loss of genetic variability at the PRL exon 5 locus. This situation could probably be attributed firstly, to the loss of gene alleles and subsequent loss of genotypic biodiversity following the massive slaughter of breeder ducks and destruction of fertile eggs during the repeated avian influenza outbreaks. Secondly, this could be attributed to the limited interbreeding of the ducks under considerable habitat loss following the devastating floods in the region. The research reminds that loss of every gene or genetic variability in the livestock populations under climate change adversities is a matter of concern as it may slow down the efforts of humanity to achieve the impending goals of food security and food safety. Therefore the study points out the urgent need of concerted efforts towards mitigation of climatic vagaries and ecosystem challenges in the breeding tracts of various indigenous species of food animals and poultry. Efficient management of the water bodies and paddy fields for the prevention of environmental degradation as well as appropriate bio-security measures to prevent disease outbreaks are recommended in the present study for the sustainable conservation of duck biodiversity in the breeding tract affected by climate-based calamities.

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