SHORT COMMUNICATION

MOLECULAR IDENTIFICATION OF *RUSA TIMORENSIS* (JAVAN RUSA) AND *RUSA UNICOLOR* (SAMBAR DEER) BASED ON MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT I (COI)

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ABSTRACT. Locally, venison is considered as a premium and exotic meat, as it is not commonly found in fresh food markets and grocery stores. Despite its limited availability, demand is always high in its niche market, especially during festive seasons which highly escalate the price. However, as an expensive delicacy, deer meat is highly susceptible to fraudulent substitution and adulteration. Authentic deer meat are currently only recognized by consumers based on their own experience, meat texture, and taste which can be quite subjective. To assist in authenticating local deer meat in the market and protect consumers from fraudsters, Polymerase Chain Reaction (PCR) analysis can be carried out to distinguish between venison and other animal meat and products. Farmed venison in Malaysia are mostly from the species *Rusa timorensis* while *Rusa unicolor* is bred in the wild. Here, we detailed a newly developed conventional PCR method that is able to detect *R. timorensis* and *R. unicolor* based on partial mitochondrial cytochrome c oxidase subunit I (COI) in a single run, thus providing a simple and more accurate alternative in venison authentication.

Keywords: Species identification, *Rusa timorensis, Rusa unicolor*

INTRODUCTION

Deer is native to Europe, Asia and North America and has been introduced and domesticated in numerous countries for centuries (Lever 1985). In 1993, Vidyadaran *et al.,* (1993) reported eight species reared in deer farms across Malaysia, namely *Cervus timorensis, Axis axis, Damma damma, Cervus unicolor, Cervus nippon, Cervus elaphus, Axis porcinis* and *Axis bawean*. Of these, *C. timorensis* comprises the majority of farmed deer population (25.6%) due to its higher reproductive performance (Vidyadaran *et al.* 1993). Until today, *C. timorensis* continues to be a popular choice for farming venison in Malaysia due to its high reproductive performance, adaptability and hardiness (Fitri, 2017; Dahlan, 2009). Based on Bancian Ternakan Rusa conducted by the Selangor State Department of Veterinary Services in 2019 and Perak State Department of Veterinary Services in 2013 (data not publicly available), all farmed cervids (for breeding, recreational or venison) in both states are *R. timorensis*. Meanwhile, native *R. unicolor* are widely bred in captivity for wildlife conservation and highly susceptible to poaching (Kawanishi *et. al.* 2014). Hence venison from *R. timorensis* and *R. unicolor* can possibly enter the market with a low price or at the highest price range triggering doubts in their authenticity.

In this preliminary study, deer from the genus *Rusa* in the *Cervidae* family available in

Malaysia, namely *R. timorensis* and *R. unicolor* were chosen as the first target group for the development of primers which are able to detect multiple cervid species.

MATERIALS AND METHOD

Samples

Eight (8) blood samples of *R. timorensis* (n=3), *R. unicolor* (n=3) and *Axis axis* (n=2) were sourced from Pusat Ternakan Haiwan Lenggong, Perak, while meat samples from deer (*R. timorensis*), chicken, goat, sheep, buffalo, cow and pig were obtained from Veterinary Public Health Laboratory (VPHL), Salak Tinggi, Selangor. 200 ul of blood samples were used directly for DNA extraction while 10 mg meat samples were grinded and mixed with 200 ul PBS before extraction. DNA extraction and purification were done using DNeasy® Blood and Tissue Kit (QIAGEN, Germany) based on the manufacturer›s protocol.

Primer design and PCR

Primers were designed based on conserved sequences in *Rusa* genus to amplify partial mitochondrial cytochrome c oxidase subunit I (COI). COI is a recognized standard used in DNA barcoding for identification of animal species (Yan, 2013). In silico, sequences of COI from *R. timorensis* (HM204510.1, KF317910.1), *R. unicolor* (HM204516.1, KF317912.1, KX156946.1), *Axis axis* (KT372098.1, MT251372.1), cow (AJ 885201), sheep (AJ 885200), goat (AJ 885199), pig (AM158316), buffalo (JQ735454.1) and chicken (JF498862.1) were obtained from the GenBank® database as reference sequences. By using Primer-BLAST algorithm, primer pair COI_F (5'-TATCGTAACCGCACATGCATT -3') and COI_R (5'-GTTTCGGTCTGTTAATAGTATTGT-3') was chosen for this study as they only amplify *R. timorensis and R. unicolor*.

PCR was carried out using DreamTaq PCR Master Mix (ThermoScientific, USA) following the manufacturer's instructions. Thermocycling conditions are as follows: 95 °C for 1 minute, followed by 40 cycles of denaturation at 95 °C for 30 sec, primer annealing at 62 °C for 30 sec and primer extension at 72 °C for 1 min. This is followed by an extension step at 72 °C for 5 min. PCR products were run on 1.5 % agarose for 30 mins at 100 V and visualized using a transilluminator. The PCR products were then sent to Apical Scientifics (M) Sdn Bhd, Malaysia for sequencing to confirm the amplicons are partial *Rusa* COI as targeted.

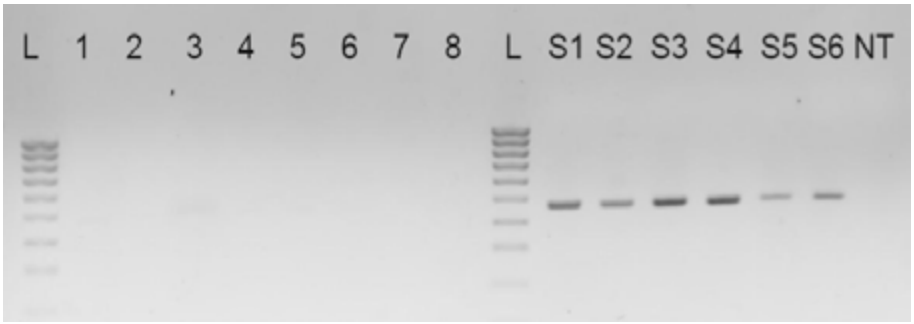

Figure 1. PCR products of COI primers. From left, lane L (100 bp ladder), lanes 1&2 (*A. axis*), lane 3 (cow), lane 4 (sheep), lane 5 (goat), lane 6 (pig), lane 7 (buffalo), lane 8 (chicken), lane L (100 bp ladder), lanes S1, S2 & S3 (*R. timorensis*), lanes S4, S5 &S6 (*R. unicolor*) and lane NT (no template control).

RESULTS

Amplicons of the targeted size (476 bp) were detected from *R. timorensis* and *R. unicolor* (meat and blood samples) while no amplification was detected from the other non-target species. Sequencing of the PCR products confirmed that partial *Rusa* COI were amplified.

DISCUSSION

Previously, no detection protocols using PCR have been established to detect and identify any Malaysian deer species by the Department of Veterinary Services VPHL laboratory although requests were received from farmers, sellers and consumers to provide authentication testing for their products and purchase. Therefore, with the PCR assay developed and described in this paper, local venison entering the market can be screened for authenticity, especially as majority of the venison are expected to be from *R. timorensis* (Dahlan, 2009). Assay specificity has also been assessed on a wider spectrum as genetic information and materials from domestic animals are included during generation of the primer and later not detected by PCR. However, more samples from *R. timorensis* and *R. unicolor* from diverse deer populations are needed to further validate this assay. A subsequent study is also needed to identify the assay detection limit to address meat adulteration issue.

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