SHORT COMMUNICATION

FERTILITY SCREENING OF BRAHMAN BULL FROM PUSAT TERNAKAN HAIWAN ULU LEPAR, PAHANG

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ABSTRACT. The purpose of this study is to evaluate the use of Breeding Soundness Examination (BSE) and sperm quality for bull fertility screening by using Brahman bulls. A total of 17 Brahman bulls were sampled from the Department of Veterinary Services' farm at Pusat Ternakan Haiwan (PTH) Ulu Lepar, Kuantan, Pahang, with age ranging between 2 to 7 years old. All candidates had undergone BSE and sperm quality test. All the data collected were analysed using SPSS software. The average age of the Brahman in this study was 55.06±14.37 months with 36.53±3.21 cm of scrotal diameter. The average libido score recorded for Brahman bulls in this study was 5.35±1.90. The minimum libido score was two (2) while the maximum score was seven (7). For sperm quality traits, the average parameter recorded for Brahman bulls were as follows: 54.41±20.15 % (sperm motility), 62.94±26.94 % (live sperm) and 49.40±8.53 % (normal sperm). The minimum and maximum score for sperm motility was 10 % and 80 %, respectively. For live sperm, the minimum score was 10 % and maximum score was 80 %. Meanwhile, normal sperm had a minimum score of 30 % and a maximum score of 65 %. The bull that fulfilled the requirements of the screening can be assigned as a superior bull for breeding program, thus making fertility screening a useful tool in monitoring potential breeder of the farm.

Keywords: BSE, sperm quality, fertility, libido, breeding programme

INTRODUCTION

Herd fertility is a projection of farm's profitability. Calving rate is one of parameters to indicate herd fertility. Bulls that are fertile are capable of conceiving many cows. According to Islam *et al.,* (2018), selection of superior bulls can be based on criteria such as physical breeding soundness, good libido and good semen quality.

Breeding Soundness Examination (BSE) is one of the tools to select superior bulls for breeding program. BSE is done twice a year in the Department of Veterinary Services' breeding herds. The bull that has successfully passed the BSE will be selected for breeding programme by introducing and rotating the bull to cow groups for a period of time for natural breeding. The selected bulls can also be utilized for artificial insemination (AI) programme. Their sperm will be collected and cryopreserved before the AI utilization in liquid nitrogen tank, which is placed at National Institute of Veterinary Biodiversity (NIVB) in Jerantut, Pahang.

MATERIALS AND METHOD

This study was conducted in September 2019. A total of 17 Brahman bulls were sampled from

the Department of Veterinary Services' farm at Pusat Ternakan Haiwan (PTH) Ulu Lepar, Kuantan, Pahang, with age ranging between 2 to 7 years old. The bulls were tested negative for reproductive diseases such as Brucellosis, Melioidosis, Johne's, Campylobacteriosis, Tuberculosis, Infectious Bovine Rhinotracheitis, Trichomoniasis, Infectious Bovine Diarrhoea and Foot and Mouth Disease. The test was in accordance with guidelines by Food and Agriculture Organization (FAO, 1990), which emphasizes on the importance of controlling transmitted diseases to ensure food safety, security, and sustainability of food production.

Bulls fertility were evaluated through BSE test, which includes the body score, general physical examination, scrotal diameter, inner and outer sex organ evaluation and bull's libido. The libido assessment was done by letting the bulls mix with cows on heat that has been synchronized with Controlled Internal Drug Release (CIDR), two weeks before the assessment. Libido score (see Table 1) was given according to the ability of the bull to stand and interact with the cow.

The sperm quality evaluation was conducted to determine the bull's sperm performance. Semen was collected by using rectal palpation technique or electro-ejaculator method. The rectal palpation method required personnel to insert a hand into the rectal and massage the internal genital organ, while the electro-ejaculator method utilized the use of a bullet probe inserted into the bull rectal to stimulate the internal genital organ via an electrical pulse. Semen quality assessment was carried out by analysing the sperm progressive motility under a dark phase microscope. Further assessments include the morphology, concentration and staining of the sperm, which were carried out in the Semen Laboratory of NIVB.

BSE data and sperm quality data were analysed by using SPSS software to obtain the fertility status of Brahman bulls. Results of libido score, scrotal diameter, sperm motility, live sperm and normal sperm are presented in Table 2.

Table 1. Bull Libido Score.

rade	Description
0	Males show no sexual reaction
1	Sexual reactions are shown only once
2	Sexual reactions are shown more than once
3	Sexual reaction always active
4	One mount without *intromission*
5	Two mounts without *intromission*
6	More than two mounts without *intromission*
7	One service was followed by no sexual reaction
8	A service followed by a sexual reaction as well as a mount without intromission
9	Two services followed by no sexual reaction
10	Two services were followed by active sexual reactions with third and more services

Table 2. Basic Statistics for Libido Score, Scrotal Diameter and Semen Quality Traits in Brahman Bulls.

BSE traits	N	Mean	Std. Deviation	Minimum	Maximum
Age, months	17	55.06	14.37	27	80
Libido score, unit	17	5.35	1.90	2	7
Scrotal diameter, cm	17	36.53	3.21	28.00	42.50
Sperm motility, %	17	54.41	20.15	10	80
Live sperm, %	17	62.94	26.40	10	80
Normal sperm, %	17	49.40	8.53	30	65

RESULT AND DISCUSSION

In this study, all samples have reached the puberty age. Randel (1994) claimed that Brahman and Brahman-based breeds matured at a later age than temperate breeds. The average age of the Brahman in this study is 55.06±14.37 months with 36.53±3.21 cm of scrotal diameter. The Society for Theriogenology (Chenoweth *et al.,* 1983) stated that the scrotal diameter must be at least 30 cm to be considered as potential sire (breeder male parent). Luis *et al.,* (2003) reported that the Brahman puberty age is 18 months with 30 cm scrotal diameter.

The average libido score recorded for Brahman bulls in this study was 5.35±1.90 %. The minimum libido score was two (2) while the maximum score was seven (7). Our result is almost similar to the finding by Hardin *et al.,* (1981) which stated that the libido score for the Brahman is 2.5. Previous studies had reported that the libido score is influenced by the production of testosterone hormone in bulls, with Brahman bulls releasing lower concentrations of testosterone than Angus bulls in their bloods (Fields *et al.,* 1982; Godfrey *et al.,* 1990).

For sperm quality traits, the average parameter recorded for Brahman bulls were as follows: 54.41±20.15 (sperm motility), 62.94±26.94 (live sperm) and 49.40±8.52 (normal sperm). The minimum and maximum score for sperm motility was 10 % and 80 %, respectively. For live sperm, the minimum score was 10 % and maximum score was 80 %. Meanwhile, the minimum score for normal sperm was 30 % and the maximum was 65 %. The differences in semen parameters among bulls may be attributed from the variations in secretary activities of the sex glands, scrotal diameter, breed, age, body size and body weight (Latif *et al.,* 2009; Islam *et al.,* 2018). Besides that, it is noted that semen quality is also influenced by the health and nutritional status of the bulls (Soeparna *et al.,* 2013).

CONCLUSION

The result of this study also indicates that BSE tool and sperm quality traits can facilitate the selection of superior bulls for breeding programmes to increase the calving rate, thus accelerating the production of meat and dairy products for local demand. It also can be noted that the variation in the data for each BSE parameter were due to many factors such as health, age, environmental condition, nutritional intake and husbandry management. For a more comprehensive assessment of fertility screening in bull, it is suggested that larger sample size is needed.

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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.

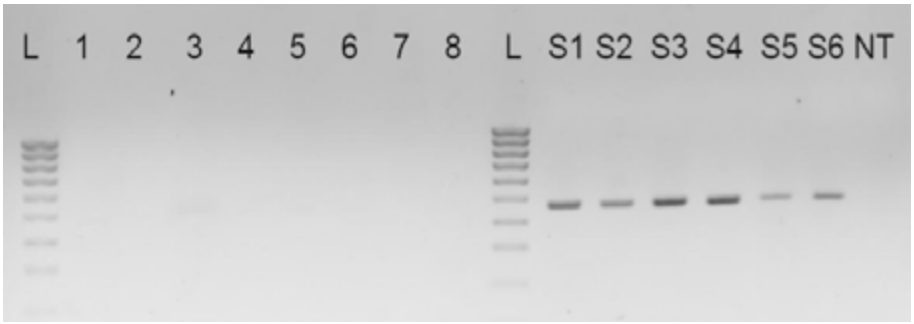

Figure 1. PCR products of COI primers. From left, lane L (100 bp ladder), lanes 1&2 (*A. axis*), lane 3 (cow), lane 4 (sheep), lane 5 (goat), lane 6 (pig), lane 7 (buffalo), lane 8 (chicken), lane L (100 bp ladder), lanes S1, S2 & S3 (*R. timorensis*), lanes S4, S5 &S6 (*R. unicolor*) and lane NT (no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