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GENETIC CHARACTERIZATION OF KEDAH KELANTAN CATTLE AND ITS CROSSBRED TYPES IN MALAYSIA

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By

HAYTHAM HAGO ABDELWAHID BASHIR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATIONS

TO MY FAMILY

WITH LOVE AND RESPECT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Professor Jothi Malar Panandam, PhD

Faculty : Agriculture

The Kedah Kelantan (KK) is the indigenous cattle breed of Malaysia and is mainly kept by small farmers for meat production because of its small and compact body, and low maintenance requirement. This breed faces risk of germplasm dilution due to extensive crossbreeding and breeds replacement practices in the country. The population size of purebred KK is fast decreasing and most of the commercial populations are actually crossbreds. There is a lack of information on the genetic characteristics of KK. The genetic relationships between the KK, the synthetic breeds developed using the KK as the maternal line, as well as the nondescriptive KK crossbred types are also unknown. It is with these in mind that the present study was conducted. The objective of the study was to evaluate the genetic variability within and among the indigenous KK cattle and the KK crossbred types in Malaysia.

DNA was extracted from the blood of 312 randomly selected animals of six cattle breed types. These included the Kedah Kelantan (KK), the Brakmas and Charoke which are synthetic breeds developed through crossbreeding of the KK, two non descriptive KK crosses (KKX1 & KKX2), and the Brahman (used as an outgroup). The breed types were assessed for 30 microsatellite loci recommended by FAO/ISAG for cattle genetic diversity studies. PCR was accomplished by using a touchdown programme. The PCR products were separated using capillary electrophoresis. Prior to screening the breed types, three genotyping methods were evaluated by comparison to the sequence information for two microsatellite loci, ETH152 and INRA005. Twelve DNA samples from KK were used for this purpose. The result indicated that the capillary electrophoresis system using labelled primers provided higher genotyping accuracy in microsatellite analysis compared to capillary electrophoresis system requiring no primer labelling primers or electrophoresis using MetaPhor agarose gel. Therefore, this method was chosen for fragment analysis in the present study.

The results showed that all 30 microsatellites loci were polymorphic for all the breed types, with 8 to 18 alleles per locus. Heterozygosity values observed for all breed types were moderate and ranged from 0.54 (KK) to 0.65 (Charoke), and were lower than the expected heterozygosity values. Seven loci (BM1818, ETH3, HEL5, HEL9, ILSTS005, INRA063 and TGLA53)

showed significant ($P < 0.05$) deviation from Hardy-Weinberg Equilibrium (HWE) for all the breed types. No breed type was observed to deviate from HWE for all 30 loci. The mean of inbreeding coefficient (F_{IS}) ranged from 0.149 (Charoke) to 0.232 (KKX2); this indicated inbreeding was present in all breed types and could lead to loss of genetic diversity if not addressed. No recent bottleneck effect was detected in any of the breed types.

The genetic distance between KK and the two KK crosses (KKX1 and KKX2) was lower than those between KK and the two synthetic breeds. Brakmas and Charoke tended to be the most distantly related. In general, the genetic differentiation measures were moderate, with a mean F_{ST} of 0.054. The analysis of molecular variance (AMOVA) indicated a greater proportion of the genetic diversity was within the breed types (96.6%) than between them. The structure analysis, phylogenetic trees, and principal component analysis showed that the breed types could be grouped into three clusters: KK and KKX2 in the first cluster, Brakmas and Brahman in second, and Charoke and KKX1 in third cluster. Structure analysis also showed that a few of the Brakmas and Cheroke animals were more KK in their genetic background; and so were many animals from the two non descriptive KK cross breed types. Analysis of zebu and taurine diagnostic alleles showed that all breed types had low proportion of African taurine and European taurine diagnostic alleles.

It may be concluded that there is still some genetic variation present in the KK and KK crossbred types. However, this genetic diversity is at risk of being lost if no appropriate breeding practices are implemented. The Charoke and Brakmas may be considered as genetically distinct breeds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN GENETIK LEMBU KEDAH KELANTAN DAN JENIS KACUKAN NYA DI MALAYSIA

Oleh

HAYTHAM HAGO ABDELWAHID BASHIR

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Pengerusi : Profesor Jothi Malar Panandam, PhD

Fakulti : Pertanian

Kedah Kelantan (KK) adalah baka lembu asli Malaysia dan kerap kali diperlihara oleh peternak kecil untuk pengeluaran daging kerana badannya yang kecil dan padat serta keperluan penyelenggaraan yang rendah. Baka ini menghadapi risiko pencairan germplasma disebabkan pengkacukan meluas dan amalan penggantian baka di negara ini. Saiz populasi KK adalah berkurangan dengan kadar cepat, dan kebanyakan populasi komersial sebenarnya adalah kacukan. Terdapat kekurangan maklumat tentang ciri genetik KK. Persaudaraan genetik antara KK, baka sintetik yang dikembangkan dengan menggunakan KK sebagai titis maternal, serta jenis kacukan KK yang *non descriptive* adalah juga tidak diketahui. Ia adalah dengan pertimbangkan perkara ini, yang kajian ini dijalani. Objektif kajian ini adalah untuk menilai kepelbagaian genetik dalam dan di antara lembu asli KK dan jenis kacukan KK di Malaysia.

DNA diekstrak daripada darah 312 ekor lembu yang dipilih secara rawak dari enam jenis baka lembu. Ini terdiri daripada baka Kedah Kelantan (KK), Brakmas dan Charoke yang adalah baka sintetik yang terhasil daripada kacukan KK, dua kacukan KK yang *nondescriptive* (KKX1 & KKX2), dan Brahman (digunakan sebagai kumpulan luar). Semua jenis baka dinilai untuk 30 lokus mikrosatelit yang syorkan oleh FAO/ISAG untuk kajian kepelbagaian genetic lembu. PCR telah dicapai dengan menggunakan satu program *touchdown*. Produk PCR telah dipisahkan menggunakan elektroforesis kapilari. Sebelum pengskrinan jenis baka, tiga kaedah penggenotipan telah dinilai dengan membandingkan kepada maklumat sekuens dua lokus mikrosatelit, ETH152 dan INRA005. Duabelas sampel DNA daripada KK telah digunakan untuk tujuan ini. Keputusan telah menunjukkan sistem elektroforesis kapilari dengan primer berlabel memberi lebih ketepatan penggenotipan dalam analisis mikrosatelit berbanding dengan sistem elektroforesis kapilari yang tidak memerlukan pelabelan primer dan elektroforesis menggunakan gel MetaPhor *agarose*. Oleh itu, kaedah ini telah dipilih untuk analisis fragmen dalam kajian ini.

Keputusan telah menunjukkan bahawa semua 30 lokus mikrosatelit adalah polimorfik untuk semua jenis baka, dengan 8 - 18 alel per lokus. Nilai heterozigositi yang telah diperhatikan untuk semua jenis baka adalah sederhana dan berada dalam lingkungan 0.54 (KK) hingga 0.65 (Charoke),

dan adalah lebih rendah daripada nilai heterozigositi jangkaan. Tujuh lokus (BM1818, ETH3, HEL5, HEL9, ILSTS005, INRA063 and TGLA53) menunjukkan sisihan ketara (P<0.05) daripada keseimbangan Hardy-Weinberg (HWE) untuk semua jenis baka. Tiada jenis baka yang didapati menunjukkan sisihan daripada HWE untuk kesemua 30 lokus. Min koefisien pembiakbakaan (F_{IS}) berjulat antara 0.149 (Charoke) hingga 0.232 (KKX2); ini menunjukkan pembiakbakaan dalam terdapat dalam semua jenis baka dan boleh membawa kepada kehilangan kepelbagaian genetic jika tidak ditangani.

Jarak genetik antara KK dan kedua-dua kacukan KK (KKX1 dan KKX2) adalah lebih rendah daripada yang di antara KK dan kedua-dua baka sintetik. Brakmas dan Charoke didapati bersaudara berpaling jauh. Secara umumnya, pengukuran pembezaan genetik adalah sederhana, dengan min F_{ST} sebagai 0.054. Analisis varians molekul (AMOVA) menunjukkan kebanyakan kepelbagaian genetik adalah dalam jenis baka (96.6%) berbanding dengan di antara mereka. Analisis struktur, pokok filogenetik, dan analisis komponen utama menunjukkan bahawa jenis baka tersebut boleh dikumpulkan dalam tiga kluster; KK dan KKX2 dalam kluster pertama, Brakmas dan Brahman dalam yang kedua, dan Charoke dan KKX1 dalam kluster ketiga. Analisis struktur juga menunjukkan bahawa sebilangan kecik ternakan Brakmas dan Cheroke adalah lebih KK dalam

latarbelakang genetik mereka; dan begitu juga banyak ternakan daripada dua jenis baka kacukan KK *nondescriptive*. Analisis alel diagnostik zebu dan taurine menunjukkan kesemua jenis baka mempunyai proporsi rendah alel diagnostik African taurine dan European taurine.

Kesimpulan boleh dibuat bahawa masih terdapat sedikit variasi genetik pada KK dan jenis kacukan KK. Walau bagaimanapun, kepelbagaian genetik tersebut adalah dalam risiko mungkin dihilang sekiranya tiada amalan pembiakan yang sesuai dilaksanakan. Brakmas dan Charoke boleh dianggap sebagai baka yang berbeza secara genetik.

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I certify that a thesis Examination Committee has met on 21th December 2012 to conduct the final examination of HAYTHAM HAGO ABDELWAHID BASHIR on his thesis entitled " Genetic Characterization of Kedah Kelantan Cattle and its Crossbred Breed Types in Malaysia" in accordance with Universities and university Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy (PhD).

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

HAYTHAM HAGO ABDELWAHID BASHIR

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