

UNIVERSITI PUTRA MALAYSIA

GENETIC CHARACTERISATIONS OF FOUR GOAT BREEDS IN MALAYSIA ASSESSED USING MICROSATELLITES

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By

AMIE MARINI ABU BAKAR

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

October 2014

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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October 2014

Chairman: Professor Tan Soon Guan, PhD

Faculty : Biotechnology and Biomolecular Sciences

The genetic characterisation of goat breeds in Malaysia as a genetic resource is essential for genetic improvement as well as a conservation strategy. The goat breeds in Malaysia comprise small-framed animals made up of the original Katjang goat with the addition of larger framed exotic goats such as Jamnapari and Boer. The local indigenous Katjang goat has a reasonably high degree of tolerance to the local environment. On the other hand, the Boer, Jamnapari and Savanna which are introduced breeds are well adapted in Malaysia.

However, little information is available on the goat breeds' genetic characteristics or genetic variabilities. Basically, information about the genetic background of a breed and its populations is required for proper breeding. Data based on adequate genetic studies for goat characterisation and their similarities are still lacking. The goat populations have to be genetically characterised because genetic information is considered as the primary and essential starting point of any goat husbandry project. Knowledge of the genetic variability of a species is a core element for the conservation and exploitation of animal diversity for breeding purposes. Thus, this study was conducted to evaluate the genetic variabilities of the goat breeds in Malaysia, namely Katjang the indigenous goat and the exotic Jamnapari, Boer and Savanna breeds, by using microsatellite markers for proper breeding, selection and conservation purposes.

Blood samples were randomly sampled from Katjang, n=37, Jamnapari, n=34, Boer, n=40 and Savanna, n=40. Thirty microsatellite primer pairs suggested by FAO/ISAG and seven microsatellite loci from other studies were used for the genetic diversity studies in the goats. The microsatellite markers were optimised using pooled DNA at different polymerase chain reaction (PCR) conditions followed by screening individuals for polymorphisms. The polymorphic microsatellite loci were used for the genetic diversity studies.

Thirty out of the 37 microsatellite loci were polymorphic in all four goat breeds, while six loci were monomorphic and one locus failed to amplify for the Boer goat breed suggesting that it was monomorphic for the null allele. The mean number of observed alleles ranged from 5.43 to 5.90. Meanwhile the observed heterozygosity values (Ho) were lower than the expected heterozygosities for the goat breeds studied. The Jamnapari and Boer goat breeds had the highest Ho (0.43), followed by the Savanna (0.42) and the Katjang showed the lowest Ho (0.36).

Genotypic linkage disequilibrium was observed between several loci but there was no consistency across the breeds. The overall mean inbreeding coefficient (F_{IS}) of 0.43 indicated the occurrence of inbreeding in all the four breeds studied. The mean F_{IT} and F_{ST} values of 0.46 and 0.06, respectively measured the degree of differentiation within and among breeds. The F_{ST} value indicated a lack of genetic differentiation among the goat breeds. The Nei's genetic identities among the four goat breeds ranged from 80% - 87%. The highest genetic distance value was observed between the Katjang and the Savanna goat breeds (0.2171) while the Boer and the Savanna goat breeds were found to be the most similar with a genetic distance value of 0.1325.

Based on this study it can be concluded that there are some genetic variations in the four goat breeds in Malaysia, although the Katjang has a slightly lower genetic diversity. However, further screening of the goat breeds, utilising more breeds and larger sample sizes are needed to ascertain more accurately the variability of the goat breeds in Malaysia. Microsatellite marker typing using the capillary technique and other more sensitive marker systems like SNP should also be considered. In addition, consideration should be given to genome mapping, using the latest high throughput genotyping platforms to obtain the best genomics solutions and applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN PENCIRIAN GENETIK EMPAT BAKA KAMBING DI MALAYSIA MENGGUNAKAN MIKROSATELIT

Oleh

AMIE MARINI ABU BAKAR

Oktober 2014

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Pencirian genetik baka kambing di Malaysia sebagai sumber genetik adalah penting untuk pembaikan genetik serta strategi pemuliharaan. Baka kambing di Malaysia terdiri daripada haiwan berangka kecil iaitu kambing asli Katjang dengan tambahan kambing eksotik berangka lebih besar seperti Jamnapari dan Boer. Kambing Katjang tempatan mempunyai darjah toleransi yang tinggi terhadap persekitaran tempatan. Sebaliknya, Boer, Jamnapari dan Savanna adalah baka yang diperkenalkan dari luar dan telah menyesuaikan diri dengan baik di Malaysia.

Walau bagaimanapun, terdapat kurang maklumat tentang pencirian genetik baka ataupun variasi genetik kambing. Pada dasarnya, maklumat tentang latar belakang baka dan genetik populasi adalah perlu untuk program pembiakbakaan yang sesuai. Pangkalan data yang mengandungi pencirian dan persamaan terhadap kajian genetik kambing masih lagi sedikit. Pencirian genetik populasi kambing perlu dikaji kerana maklumat genetik ini dianggap sebagai titik permulaan dan penting dalam projek penternakan kambing. Maklumat variasi genetik pada sesuatu spesis merupakan elemen utama untuk pemuliharaan dan mengeksploitasi kepelbagaian genetik bagi tujuan pembiakbakaan. Oleh itu, kajian ini dijalankan untuk menilai variasi genetik baka kambing di Malaysia, iaitu kambing asli Katjang dan baka eksotik Jamnapari, Boer dan Savanna, dengan menggunakan penanda mikrosatelit untuk kegunaan pembiakbakaan, pemilihan dan pemuliharaan yang sewajarnya.

Sampel darah diambil secara rawak daripada Katjang, n=37, Jamnapari, n=34, Boer, n=40 dan Savanna, n=40. Tiga puluh pasangan primer mikrosatelit yang dicadangkan oleh FAO/ISAG dan tujuh lokus mikrosatelit yang dilaporkan daripada kajian lain telah digunakan untuk kajian kepelbagaian genetik pada kambing. Penanda mikrosatelit dioptimasikan menggunakan gabungan DNA pada keadaan PCR yang berbeza diikuti oleh pemeriksaan polimorfik terhadap individu. Lokus mikrosatelit yang polimorfik digunakan untuk kajian kepelbagaian genetik.

Tiga puluh daripada 37 lokus mikrosatelit adalah polimorfik dalam kesemua empat baka kambing, manakala enam lokus adalah monomorfik dan satu lokus gagal diamplikasi untuk baka kambing Boer yang mencadangkan bahawa ia adalah monomorfik untuk alel tidak hadir. Purata bilangan alel yang dicerap berjulat daripada 5.43 kepada 5.90. Manakala nilai heterozigositi yang dicerap (Ho) adalah lebih rendah berbanding heterozigositi yang dijangka pada baka kambing yang dikaji. Baka kambing Jamnapari dan Boer mempunyai Ho yang tinggi (0.43), diikuti dengan Savanna (0.42) dan Katjang menunjukkan Ho yang terendah (0.36).

Ketakseimbangan rangkaian genotip dijumpai antara beberapa lokus tetapi ianya tidak konsisten pada seluruh baka. Keseluruhan purata pekali pembiakbakaan dalam (F_{IS}) adalah 0.43 menunjukkan kewujudan pembiakan dalam pada semua empat baka yang dikaji. Nilai purata F_{TT} dan F_{ST} adalah 0.46 dan 0.06, masing-masing diukur pada tahap perbezaan di dalam dan di antara baka. Nilai F_{ST} menunjukkan kekurangan perbezaan genetik di antara baka kambing. Kesamaan genetik Nei antara empat baka kambing berjulat di antara 80% - 87%. Nilai jarak genetik yang tinggi diperolehi di antara baka kambing Katjang dan Savanna (0.2171) manakala baka kambing Boer dan Savanna dijumpai mempunyai kesamaan berdasarkan nilai jarak genetik iaitu 0.1325.

Berdasarkan kajian ini ia dapat disimpulkan bahawa terdapat variasi genetik dalam empat baka kambing di Malaysia, walaupun Katjang mempunyai kepelbagaian genetik yang lebih rendah sedikit. Walaubagaimanapun, saringan lanjutan terhadap baka kambing dengan menggunakan lebih banyak baka dan bilangan haiwan yang lebih banyak diperlukan untuk menentukan dengan lebih tepat variabiliti baka kambing di Malaysia. Penanda mikrosatelit menggunakan teknik kapilari dan sistem penanda genetik yang lebih sensitif seperti SNP juga perlu dipertimbangkan. Disamping itu, pertimbangan harus diberikan kepada pemetaan genom, menggunakan hasilan tinggi yang terkini terhadap platform genotip untuk mendapatkan penyelesaian genomik dan aplikasi yang terbaik.

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I certify that an Examination Committee has met on 21 October 2014 to conduct the final examination of Amie Marini Abu Bakar on her Master of Science thesis entitled "Genetic Characterisations of Four Goat Breeds in Malaysia Assessed Using Microsatellites" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Masters of Science.

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LIST OF ABBREVIATIONS

AFLP	-	amplified fragment length polymorphism
bp	-	base pair
°C	-	degree celsius
cm	-	centimetre
df	-	degree of freedom
ddH ₂ O	-	double distilled water
DNA	-	deoxyribonucleic acid
dNTP	-	deoxyribonucleotide triphosphates
DVS	-	Department of Veterinary Services
EDTA	-	ethylenediaminetetraacetic acid
FAO	-	Food and Agriculture Organization
FIS	_	inbreeding coefficient
F _{IT}	-	deficiency or excess of average heterozygotes in a group of
R.		population
Γ _{ST}	-	degree of gene differentiation among populations
g	-	gram
He	-	expected heterozygosity value
HO	- T	observed heterozygosity value
HWE	1	Hardy-weinberg equilibrium
ISAG	7	International Society for Animal Genetics
Kg	-	kilogram
	-	linkage disequilibrium
MARDI	-	Institute
mg	-	miligram
MgCl ₂	-	magnesium chloride
ml	-	millilitre
mM		milimolar
mm	- ^	millimetre
μl	-	microlitre
μg	- 6	microgram
μM	-	micromolar
MoDAD project	-	The Global Program for the Management of Farm Animal
		Genetic Resources Project
Ne	-	effective number of alleles
Na	-	observed number of alleles
ng	-	nanogram
nm	-	nanometre
PAGE	-	polyacrylamide gel electrophoresis
PCR	-	polymerase chain reaction
RAPD	-	random amplification of polymorphic DNA
RFLP	-	restriction fragment length polymorphism
S	-	second
SNP	-	single nucleotide polymorphism
SSR	-	simple sequence repeats
STR	-	short tandem repeat
TAE	-	tris-acetate EDTA

TBE	-	tris-borate EDTA
U	-	Unit
UPGMA	-	unweighted pair group method with arithmetic mean
UV	-	ultraviolet
V	-	Volt
VNTR	-	variable number tandem repeat
1X	-	one time concentration
x g	-	relative centrifocal force



CHAPTER 1

INTRODUCTION

The goat population in Malaysia has increased over the years since 2000. In Peninsular Malaysia, the total goat in 1999 was 190,539, then it increased to 373,319 in 2007 and 413,359 in 2011. Meanwhile, the total goat in Malaysia, including East Malaysia (Sabah and Sarawak) was about 458,646 (DVS, 2010, 2011). However, increasing the number of goats are still not be able to supply enough chevon for the Malaysian consumers. The demand for chevon and mutton has steadily increased. The self-sufficiency rate for goat meat and mutton in 2011 was estimated at only 11.28% (DVS, 2011). The chevon and mutton industry in Malaysia needs to be further increased in order to reach the targeted self-sufficiency rate of 35% by 2015. The demand of chevon was met through importation of meat and live animals from Australia and New Zealand which has increased annually.

At present, there are about 4500 farmers and entrepreneurs rearing goats and sheep in Malaysia (Wan Zahari *et al.*, 2008). Most of the goats and sheep are raised by small farmers (75%) and the rest pursued by commercial farmers (20%) and integration projects (5%). The Department of Veterinary Services (DVS) has a number of farms, in particular to carry out breeding of goats and sheep. Encouragement from the government to increase the population through the importation of goat and sheep breeds that are highly productive were the main strategies for developing the nation's goat industry. This effort expanded to private sector involvement in commercial breeding.

In commercial farming, high quality breeds must be available at the right time and in sufficient quantities. Therefore, there is great interest in goat farming in Malaysia. Improving the quality of livestock breeds and increasing animal productivity to maximise profit also is an important strategy in the development of the goat industry. The advances in biotechnology have been implemented to improve livestock production through breeding and selection. Reducing importation of live goats and chevon is necessary to improve the goat production in Malaysia. The characterisation of the breeds is important for improving the genetic performance and to decrease inbreeding.

One of the key drawbacks of the goat industry in Malaysia is the lack of proper breeding and selection. Basically, information about the genetic backgrounds of breeds and populations is required for proper breeding. Database on genetic studies on goat characterisation and comparison of breeds is lacking. The goat breeds and populations have to be genetically characterised because is considered as a primary and essential starting point of any goat genetic improvement project. The genetic characterisation of goat breeds in Malaysia as a genetic resource is essential for genetic improvement as well as a conservation strategy. The goat breeds in Malaysia comprise of small-framed animals made up of the indigenous Katjang goat with the addition of larger framed exotic goats such as Jamnapari and Boer. The Katjang goat has acquired a reasonably high degree of tolerance to the local environment. The Boer, Jamnapari and Savanna which are introduced breeds have become well adapted in Malaysia. Nowadays, pure breed of the indigenous Katjang goat is difficult to find (Department of Veterinary Services, 2006). Most of the Katjang goat was crossbred with introduced breeds, particularly with Jamnapari goat. Currently, these introduced breeds are popular among farmers as high meat producers. They are been imported and bred in Malaysia to fulfill the industry's demand. However, disorganised and uncontrolled crossbreeding activities among farmers have contributed to inbreeding and admixture (Panandam, 2007). At the same time, the indigenous Katjang goats were been neglected and overlooked in breeding effort. Hence, special priority must be given to plan and execute strategic breeding programme, and to conserve the different goat breeds.

Thus, breed characterisation is the first step in developing programmes for sustainable utilisation and conservation. Breed or genetic characterisation can be carried out using molecular techniques. Numerous studies have been initiated to characterise goat breeds using molecular techniques. Microsatellite markers can be used to characterise animal species, breeds as well as for establishing genetic relationships among or within species (Kim *et al.*, 2004; Sunnucks *et al.*, 2001). Establishment of genetic relationships among the goat breeds will help in genetic improvement and conservation of the goat breeds in Malaysia.

1.1 Problem Statement

There are very limited documentations and published reports on genetic variations of the goat breeds in Malaysia particularly in the indigenous Katjang goats. The Jamnapari goats that have been brought in long ago, to be used for natural and/or artificial selection and local breeding practices are well adapted to the local environmental condition and production system. However, due to improper breeding practice, lack of knowledge in animal breeding principles and poor record keeping contributed to the loss of local breeds. Thus, it is essential to assess the genetic characteristics and variability of the goat breeds in Malaysia which include local and introduced breeds. Conservation of local animal genetic resources is only possible through genetic characterisation, sustainable utilisation and management. Furthermore, introduced breeds such as Boer and Savanna goats well adapted to the hot tropical climate in Malaysia should be evaluated and monitored for proper breeding utilisation.

1.2 Objective

The general objective of this study was to determine the genetic variability of goat breeds in Malaysia using microsatellite markers.

The specific objectives were:

- 1. To evaluate the genetic polymorphism of goat breeds in Malaysia
- 2. To establish the genetic distance and relationships between the indigenous Katjang goats and the introduced exotic goat breeds.

1.3 Research Hypothesis

The local Katjang goats originated from Malaysia and Indonesia. However, pure local Katjang goats are difficult to find since most of them have been crossed with introduced breeds. Meanwhile Jamnapari goats which originated from India were brought to and bred in Malaysia a long time ago. Genetically, Katjang and Jamnapari goats should differ in their genetic makeups, but may share similarities as the results would be influenced by their histories. On the other hand, Boer and Savanna goats which originated from South Africa and Australia, respectively, were recently introduced and developed in Malaysia. Therefore, Boer and Savanna goats should differ in their genetic structures. The genetic distance might reveal the Katjang and Jamnapari to be from the same cluster because of admixture with each other. Meanwhile, the Boer and Savanna could be in a similar group because of same origin.

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