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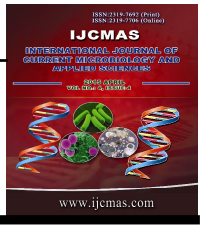

Original Research Article

Assessment of Genetic Diversity in Saudi Goats, Saudi Arabia Using Genetic Finger printing

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Keywords

Genetic Diversity, Saudi Goats, Saudi Arabia, Genetic Fingerprinting

A B S T R A C T

Goats are considered one of the important economic sources of meat in developing countries. Improving the reproductive efficiency of goat herds in these countries, can increase the efficiency of progeny production and consequently goat meat. Thirty animals were randomly selected from three Saudi goat breeds namely, Najdi, Harri and Aradi. Ten animals per breeds were selected, and blood samples were collected from total of 30 animals into EDTA tubes. Genomic DNA was extracted from blood according to DNA preparation kit to determine the genetic relationship among studied goat animals. Four different primers were used in RAPD - PCR reaction. The results showed that the total scored bands using the four primers with the three goat breeds were 1071 PCR bands among 136 amplified bands. Out of which 63 bands were polymorphic (46.32%) and 73 bands were monomorphic (53.67%), respectively. We conclude that RAPD- Fingerprinting is a simple and fast molecular technique to evaluate polymorphism in goat breeds.

Introduction

Goat (*Capra aegagrus*), as one of the ancient livestock, was first domesticated in Western Asia in the period of 9000 - 7000BC (Zenuner 1963). It was considered that the domestic goat breeds in the world were from a single origin and their separations in different ecological areas (Nozawa 1988). Zhang and Li (1990) suggested that the goat in the world could be divided into three populations according to the Hb gene frequency (Li et al., 2004).

Generally, goat breeds are named by their places of origin and classified by their uses of products. Asthe effect of different ecological conditions and long-term artificial selection, goat breeds with different genetic characteristics and product orientation are formed (Yue 2000).The largest number of goats is observed in Asia, followed by Africa, representing about 59.7% and 33.8%, summing up to 93.5% out of the total number of the world, Dairy goats

produce about 15.2 million metric tons (MT) of milk, accounting for about 2% of the world total amount of milk produced by livestock species (FAOSTAT, 2008). Goats are one ofthe world's most adaptable and widespread livestock species, and are one of the main economic recourses in many developing countries (Luikart et al., 2001).In Saudi Arabia where the climate is suitable for goats, the number of these animal is believed to exceed 2.5 million (Salah et al., 1989) mainly of the Masri (Egyptian) and Ardi (Baldi) breeds. Also, there are two other breeds, however, with less numbers (Harri and Habsi). The Ardi goats are more adapted to the arid region than Masri. Although, the latter produces more milk (Al Saidy et al., 2007) while the first produces milk steadily and therefore, is greatly appreciated by desert dwellers, where it is widely spread. The contribution of goat as a source of meat to total meat income of Saudi Arabia is about 30%.Recent advances in DNA molecular technology have provided new opportunities to assess genetic diversity at the DNA level (Vostry et al., 2011). ISSR markers has currently been one of the most useful marker of choice for a wide range of molecular genetic studies such as establishing population structure, population differentiation and reconstruction of phylogenetic relationships among populations specially in plants (Wang et al., 2008). In this method, microsatellite sequences of DNA are used as primers for comparison the length of the sequences amplified between them. These markers allow moving from traditional studies of single loci polymorphism (structural genes, microsatellite loci) to an analysis of multiloci spectra representing polymorphism of many genomic fragments. The use of microsatellite loci as primers in PCR to reveal polymorphism of various sites of genomic DNA is based on the act that microsatellites present in a genome with very high frequency and ISSR method marking suchpolymorphisms lead to reveal multiloci and polymorphic spectra of genomic fragments (Glazkoet al.,al 2009). Triapitsyna and Glazko (2005) studied thegenetic structure changes and polymorphism peculiarities of Holstein cattle that reproduced under influence of low-dose irradiation of Chernobyl accident. Some other similar studies have been taken on cattle and sheep spices in recent years (such as Zamani et al., 2009; Stolpovsky et al., 2010), however, to our best knowledge, the studies of ISSR marker in Goat specie is very limited. Yong et al. (2010) and Askari et al. (2011) studied the genetic diversity of Ritu Tibetan Goats and Rayini goat using this marker, although the sequence of primers used in these studies are different to our research.

The objective of this study was to evaluate the genetic diversity in Saudi goat using RAPD fingerprinting.

Materials and Methods

Sampling

Thirty animals were randomly selected from three Saudi goat breeds, 10 animals per breeds were selected (5 males and 5 females), namely, Najdi (n:1-5 males , n: 6-10 females),Harri (n: 11-15 males, n: 16-20 females) and Aradi (n: 21-25 males . n: 26-30females). ,

DNA Extraction and PCR Amplification:

Blood samples were collected from 30 animals into vacatinrswith EDTA as anticoagulant. Samples were kept at -20°C until use. Genomic DNA was extracted from peripheral blood lymphocytes according to instructions of blood DNA preparation kit (Jena Bioscience, Germany).

Random amplified polymorphic DNA (RAPD)

Four different primers were used in PCR reaction which consists of 10 pmol of each different arbitrary 10-mer primers and 25 to 50 ng of genomic DNA and 12.5 µl of 2x SuperHot PCR Master Mix (Bioron, Ludwigshafen, Germany). The names and sequences of these oligoprimers are listed in Table 2. The RAPD-PCR amplification reactions were performed in Eppendorf® thermal cycler using the following PCR program: 1cycle at 94°C, 4 min; 35 additional cycles consisting of 94°C 5 s, 37°C 20 s and 72°C 20 s. After the amplification, the PCR reaction products were electrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 x 14 cm 1.5%-agarose gel (Bioshop; Canada) for 30 min using Tris-borate- EDTA Buffer. The gel was stained with 0.5 µg/ml of ethidium bromide (Bioshop; Canada).

Determination of genetic relationship

In order to determine the genetic relationship among studied goat animals RAPD data were scored for presence (1) or absence (0) of the bands. The data were transferred to a statistical software program, Statistical Package for Social Science, version 10.00 (SPSS Inc, Chicago, Illinois, USA) to obtain analytical statistics in the form of Jaccard's similarity coefficient (S) showing the genetic similarity among different examined bacterial isolates based on pair-wise comparison. The dendrogram was constructed using the Average Linkage between groups.

Analysis of the PCR products

RAPD amplified fragments were scored for band presence (1) or absence (0) and a binary qualitative data matrix was constructed. Data analysis was performed using the NTSYS PC version 2.02 computer package program(Rohlf, F.J., 2000.). The similarity values were used to generate a dendrogram via the un- weighted pair group method with arithmetic average (UPGMA). Measurement of diversity including gene diversity (H), observed number of alleles (Ne), gene flow and Shannons information index were estimated by POP- GEN 3.2 software.

Result and Discussion

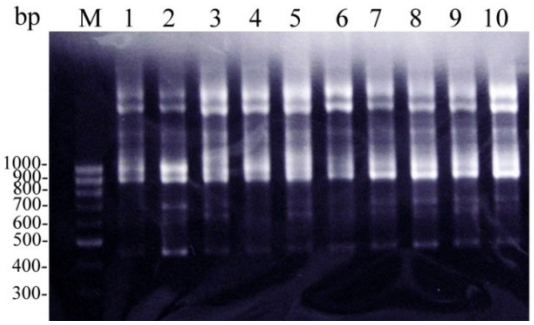
To ensure that the amplified DNA bands originated from genomic DNA, and not primer artifacts, negative control was carried out for each primer/breed combination. No amplification was detected in control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions. All primers (OPA-1, OPA-2, OPA-3, and OPA-4) were successfully amplified polymorphic bands among the three breeds studied (Fig.1- 4).The total number of produced bands and scored percentages of polymorphism for each primer among studied goat breeds were illustrated in Table 1. Each used primer produced informative electrophoretic profile.

The total scored bands for each primer (OPA-1, OPA-2, OPA-3, and OPA-4) with three goat breeds were 288. 276. 197. And 314, respectively, while the total scored bands for each breed goat using the four primers were 426, 383, and 262, respectively, and the total scored bands using the four primers with the three goat breeds were 1071 PCR bands among 136 amplified bands. Out of which 63 and 73 bands were polymorphic (46.32%) and monomorphic (53.67%), respectively.

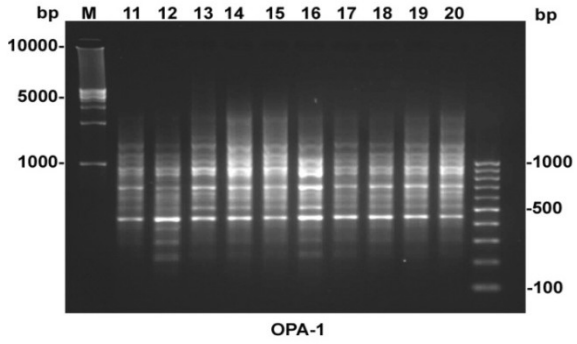
Table.1 List of primers code, sequences, % and average of polymorphism and number of produced bands among three studied goat breeds

Primer code	Primer sequence	breeds			Total scored bands	Amplified bands	Polymorphic markers	Monomorphic markers	Polymorphism %
		Najdi	Harri	Arradi					
OPA1	CAGGCCCTTC	82	133	69	**288**	33	9	24	27.3
OPA2	TGCCGAGCTG	99	110	67	**276**	37	20	17	54
OPA3	AGTCAGCCAC	127	34	36	**197**	26	14	12	53.8
OPA4	AATCGGGCTG	118	106	90	**314**	40	20	20	50
Total		**426**	**383**	**262**	**1071**	**136**	**63**	**73**	

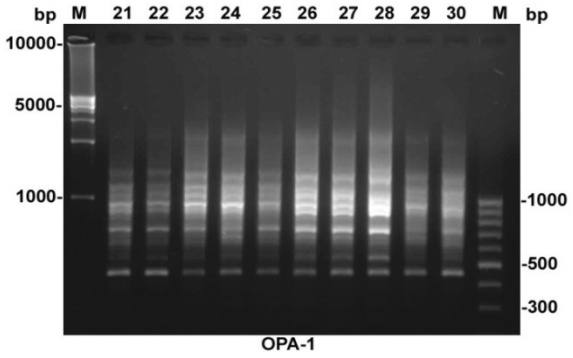
Fig.1 RAPD profiles obtained with primer OPA-1 from the DNA of Najdi (n:1-5 males, n: 6-10 females) Harri (n: 11-15 males, n: 16-20 females) and Aradi (n: 21-25 males, n: 26-30 females). Lane 1 M; molecular size marker (100 bp DNA ladder)



OPA _ 1



OPA-1



OPA-1

Fig. 2: RAPD profiles obtained with primer OPA-2 from the DNA of Najdi (n:1-5 males, n: 6-10 females) Harri (n: 11-15 males, n: 16-20 females) and Aradi (n: 21-25 males, n: 26-30 females). Lane 1, M; molecular size marker (100 bp DNA ladder)

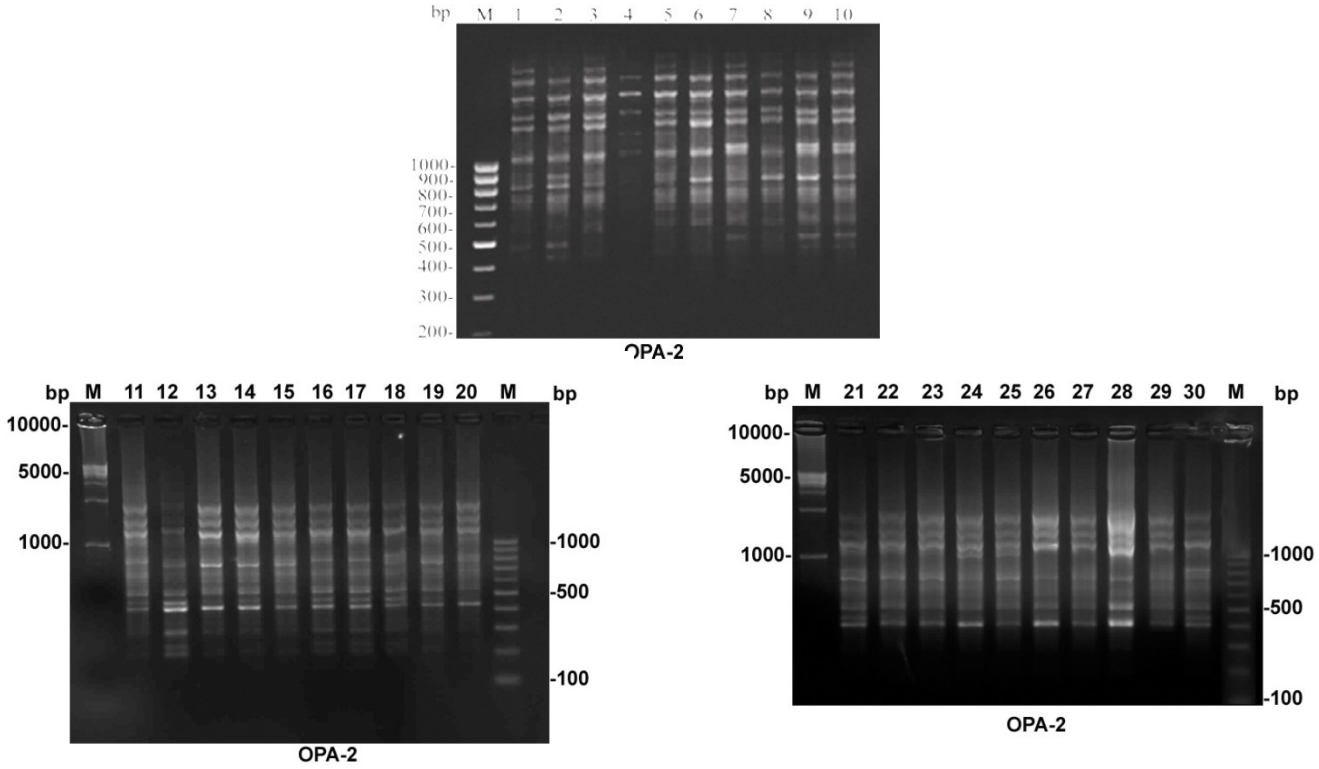


Fig.3 RAPD profiles obtained with primer OPA-3 from the DNA of Najdi (n:1-5 males, n: 6-10 females) Harri (n: 11-15 males, n: 16-20 females) and Aradi (n: 21-25 males, n: 26-30 females). Lane 1 M; molecular size marker (100 bp DNA ladder)

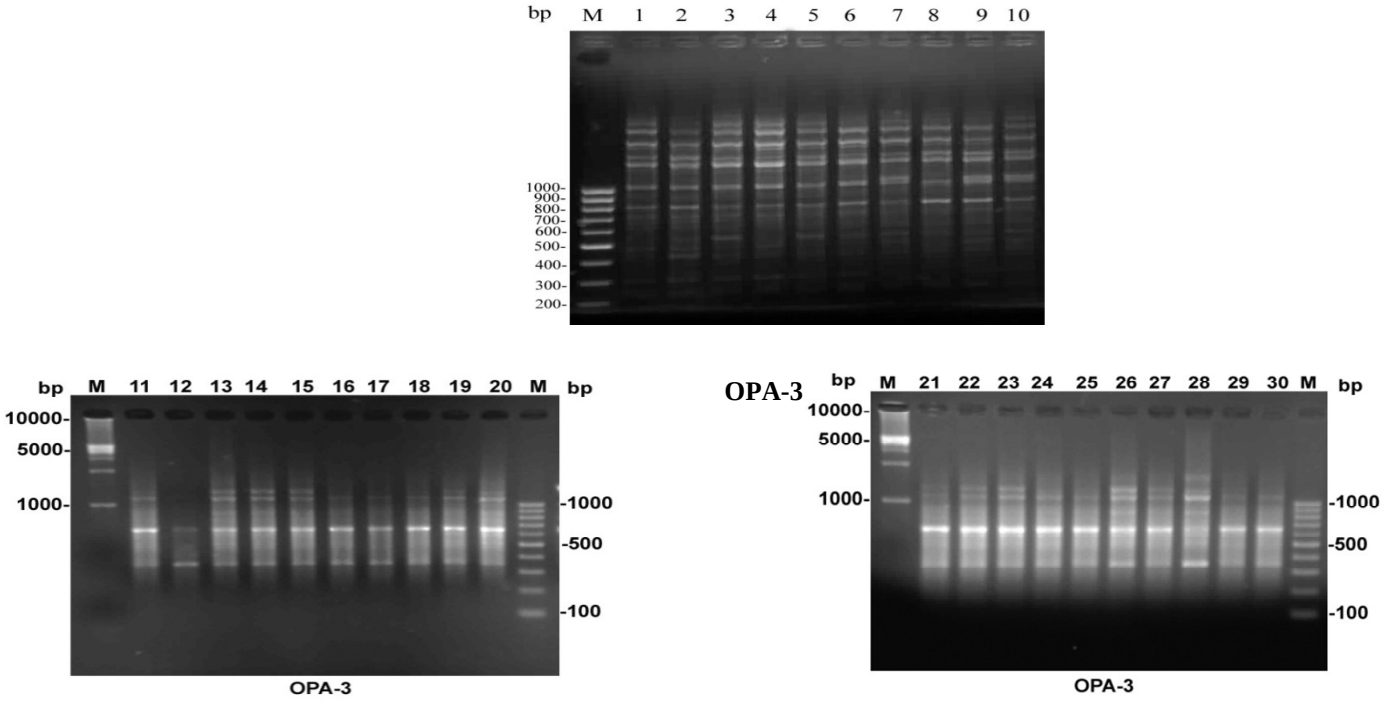


Fig.4 RAPD profiles obtained with primer OPA-4 from the DNA of Najdi (n:1-5 males, n: 6-10 females) Harri (n: 11-15 males, n: 16-20 females) and Aradi (n: 21-25 males, n: 26-30 females). Lane 1 M; molecular size marker (100 bp DNA ladder)

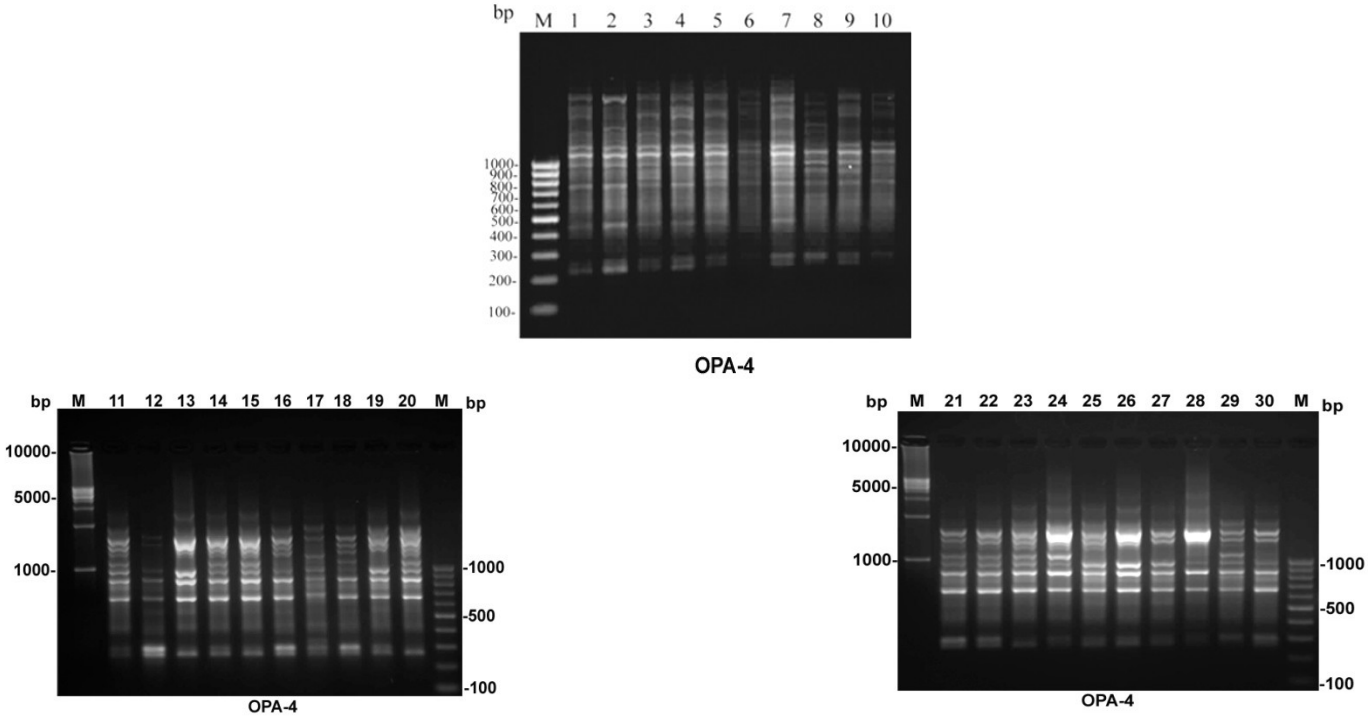
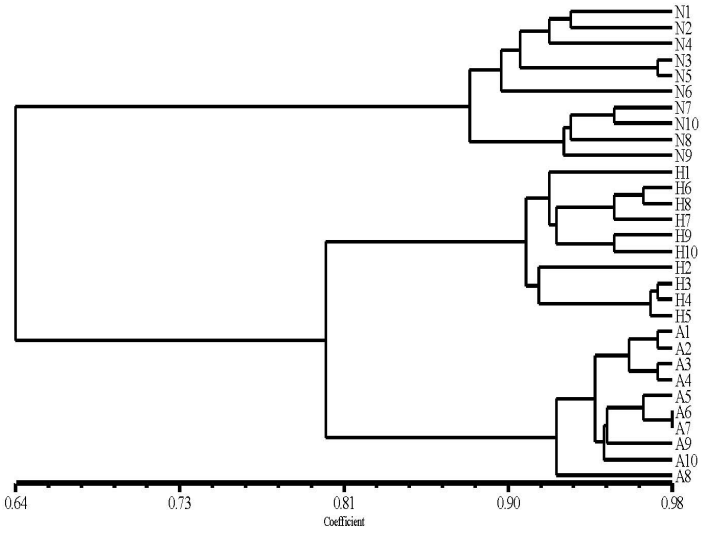


Fig.1 Phylogenetic relationship and genetic distance within and between studied Najdii (N1-N10), Harri (H1-H10) and Aradi (A1-A10) goat breeds with UPGMA dendrogram based on Jaccard's coefficient



The number of scored band per each animal in different breed ranged from 6.56 bands to 10.46 bands, while the amplified bands per each primer ranged from 26 to 40 bands, in addition the percentage of polymorphism per each primer ranged from 27.3 to 54 %.

RAPD analysis was used for constructing parsimony tree depicting relationships among the three breeds. The resulted dendrogram in the Figure 2 showed that, there are three main separate clades. Each 10individuals belongs to the same breed were clustered together. The first clade includes (1-10 Najdi individuals).While the second comprised from (11-20) Harri individuals and the third clad includes (21-30) Araadi individuals. We found that the genetic similarity between first clad individuals was the lowest (88%) representing Najdi breed, while the third clad was the highest (93%) representing Araadi. However, the second clad was the medium (90%) representing Harri individuals.

Variation of size and number of amplified fragments between and within breeds could be a result of nucleotide changes at the primer annealing site or due to deletion or addition between two priming sites (Sharma et., el, 2001). Also, these variations illustrate the different pattern of amplification of primers. Furthermore, it suggests the genetic heterogeneity between and within breeds. It also, explored the ability of RAPD primers to investigate the genetic polymorphism among studied breeds. RAPD markers employed in this study assured to be a good technique in differentiation between individuals belong to different breeds (Ali, 2003), Kumar (2008), Elmaci et., al 2007). Each breed (10 samples clustered together in one separate clade) illustrated the fact that each sample belongs to the same breed and there was a high similarity between individuals (Khaldi et., el. 2010), Halder and Shriver 2003). This results suggested high percentage of homogeneity within each breed and high level of genetic variability among studied breeds. The noticed interbreed variability might be due to a difference in the population, individual variations, and architecture and/or might be due to genetic stratification (Ziv, and Burchard 2003, Jawasreh 2011).

In Conclusion, the results described in this paper demonstrated that the technique of RAPD is short of stability, but it is a simple and convenient method, producing much polymorphic information of target genomes with more primers can compensate the coming of this method. The technique of RAPD is still a good method of studying genetic variation with breed and genetic distance among breeds.

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