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Cytogenetic Profile of Rajapalayam Dog Breed of Southern India

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ABSTRACT

Rajapalayam, an important dog breed of southern India which are maintained for guarding of farm houses and protection from wild animals. The aim of the present investigation is to study the karyology of this important dog population for cytological profiling. Metaphase plates were prepared after culturing of lymphocytes isolated from heparinized blood collected from animals of both the sexes. Giemsa banding, centromeric index, arm ratio and relative length were estimated through standard protocol. The cytogenetic profile of Rajapalayam dog is different from that of Chinese Raccoon and Japanese Raccoon dogs which belongs to Canidae family with a basic chromosome number of 78 without B chromosome. The X chromosomes are longer in bitches than that of in dogs. Giemsa banding analysis revealed higher number of bands (272) distributed among the 38 chromosome in Indian dog breed as compared to that Chinese Raccoon dog. The results of the present study gave insight knowledge to the researchers, dog breeders and kennel clubs about the karyology of Indian dog breeds and to know about any chromosomal abnormalities which may leads to fertility, growth and phenotypic abnormality related problems in this dog breed.

Key words: G banding, Karyotype, Rajapalayam dog.

INTRODUCTION

Dog (*Canis lupus familiaris),* the first domesticated animal and has been most widely kept as guarding, hunting and pet animal, belongs to subspecies of gray wolf (*Canis lupus*), a member of Canidae family of the mammalian order Carnivora. Apart from 160 registered livestock breeds of various species there are several breeds of dogs in India viz., Caravan Hound, Combai, Chippiparai, Rajapalayam, Rampur Hound, Kanni, Mudhol Hound, Indian Mastiff (Bulli), Himalayan sheep dog, Bhutia dogs etc,. Dog, particularly the exotic breeds are companion animals in recent days in urban areas but the Indian breeds are mainly utilized for guarding the farm and farm houses and for shepherding the livestock species during grazing, migration and hunting. Animal cytogentic studies may be helpful in identifying the chromosomal abnormalities which may lead to reproductive failures or infertility related problems particulary in males. Chromosome analysis in dog may pave way to test the dogs for karyological anamolies which is key for dog breeding. Puppies are of great demand for Rajapalayam dog because of it guarding behavior. So far adequate attention has not been given to cytogenetic characterization of Indian dog breeds, cytogenetic profiling in dogs are of great value especially for breeders dealing with fertility problems within their pedigrees, for veterinarians and for the dog owners. No information is available on Rajapalayam dog karyological profile hence; the present study was undertaken with the objective of cytogenetic profiling of Rajapalayam dog breed of southern India.

MATERIALS AND METHODS

Peripheral blood samples were collected in heparinized vials with aseptic precautions from 25 adult Rajapalayam dog (15 males and 10 females) maintained by various dog owners of Madurai, Virudhunagar, Thoothoukudi and Tirunelveli districts of southern Tamil Nadu, India. Short-term peripheral blood lymphocyte culture technique (Moorhead *et al.* 1960) was followed with few modifications. The cultures were set up in a sterile 15 ml culture tube by placing 0.2 to 0.3 ml of white blood cells (buffy coat) in 8 ml of RPMI 1640 medium supplemented with L-glutamin, 1.5 ml foetal calf serum and 100 mg of Phytohaemagglutinin (PHA) mitogen. The cells were cultured by incubation at 37° C for 72 hours. The cells were harvested by the addition of colchicine at 70.5 hours. The cell suspension was incubated in hypotonic solution (0.075 M potassium chloride) for 20 minutes and fixed in methanol and acetic acid (3:1). Metaphase spreads were prepared in a clean pre-chilled glass slide and air dried.

Banding of chromosomes: G-banding of chromosomes was carried out using trypsin digestion protocol (Barch *et al.* 1991). C-banding technique was followed by using barium hydroxide (Sumner *et al.* 1971). The slides were viewed under microscope (Carl Zeiss) and good metaphase spreads were photographed for developing banded karyotypes.

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Centromeric Index, Arm Ratio and Relative length: The centrometric index, CI_1 was calculated as the ratio of length of long arm to short arm. Arm ratio was calculated as ratio between the length of long arm and short arm. The relative length was calculated as the length of the whole chromosome to the total length of all chromosome in haploid set.

$$CI\ (\%) = \frac{\text{The length of the shorter arm of the two chromosome arms}}{\text{The length of the whole chromosome}} \times 100$$

$$\text{Arm ratio} = \frac{\text{Length of long arm (L)}}{\text{Length of short arm (S)}}$$

$$\text{Relative length}\ (\%) = \frac{\text{Length of the whole chromosome}}{\text{Total length of all chromosome in haploid set}} \times 100$$

RESULTS AND DISCUSSION

Karyotype analysis: Chromosome analysis of Rajapalayam breed of dogs revealed the common fundamental number (2n = 78), with 38 pairs of acrocentric autosomes, one large sub metacentric X chromosome and a small sub metacentric Y chromosome (Figure 1a & 1b). The size of the acrocentric chromosomes gradually decreased from the first pair to thirty eighth pair of autosomes in both the sex (Figure 2). The findings were in agreement with the reports on chromosome analysis of domestic dogs (Suryawanshi *et al.* 2004). The male and female Karakachan dog (Bulgarian native dog) have chromosome number 78 (2n). At the basic cytogenetic level, the Karakachan dog karyotype consists of 38 pairs of acrocentric autosomes gradually diminishing in size. Sex chromosome pair is represented by two large metacentric X in females. The Y chromosome in males has metacentric morphology and is the shortest one in the complement

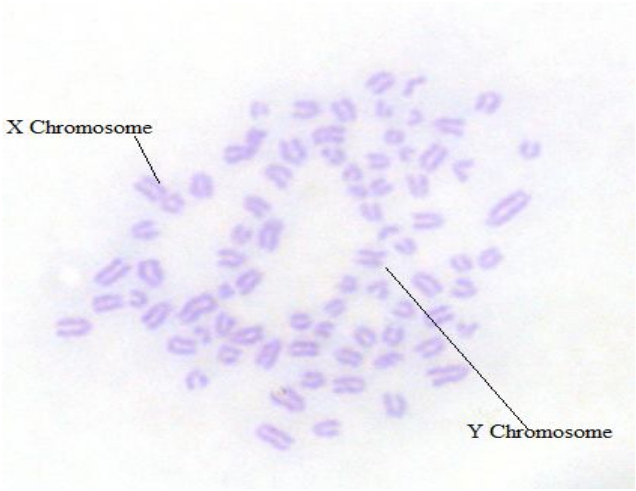

Figure 1 a: Metaphase spread of Rajapalayam dog

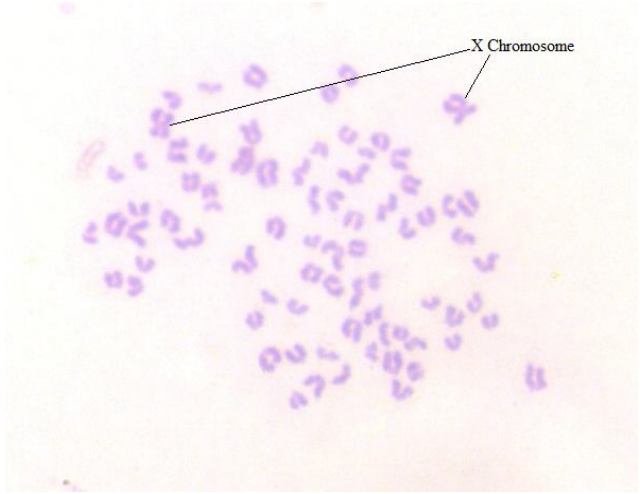

Figure 1 b: Metaphase spread of Rajapalayam bitch

(Topashka *et al.* 2009). The karyotype of the Chinese Raccoon dog (2n = 54 + B) is composed of five pairs of bi-armed autosomes and 21 pairs of acrocentric autosomes. The sex chromosomes are also bi-armed, a medium-sized X and the Y being the smallest chromosome in the karyotype (Switonski *et al.* 2003).

The mean mitotic index in the slides prepared by dropping the cell suspension was found to be 2.78 ± 0.20 per cent. The longest autosome contributed 4.06 and 3.58 per cent and the smallest about 1.43 and 1.52 per cent to total chromosomal complement in male and female animals respectively. The X chromosomes accounted for 4.64 and 3.73 per cent in males and females, respectively, while the Y chromosome accounted only 1.38 per cent. The mean relative length of chromosomes of male and female Rajapalayam dogs were gradually diminished from the first pair to the last pair of autosomes, as depicted in Figure 3. The first pair of chromosome had a length of 7.92 ± 0.02 and 10.8 ± 0.24 mm in males and females, respectively. Whereas, the last pair of autosomes had 2.77 ± 0.03 and 4.57 ± 0.03 mm in males and females, respectively. The X chromosome was observed to be 11.30 ± 0.06 mm length in bitch, whereas, in dog it was 9.10 ± 0.06 mm length, indicated that the X chromosome is longer in females than that of male dogs. As reported, the Japanese raccoon dog karyotype (2n = 38 + B) comprised of 13 pairs of bi-armed chromosomes, 5 pairs of acrocentric chromosomes and bi-armed X and Y chromosomes. In this sub-species, a variable number (2 to 7) of B chromosome is also present (Wada and Imai, 1991; Wada, 1998). The dog breed under the study does not revealed any B chromosome in the Karyological analysis, which was a distinct observation in Rajapalayam dog breed.

Giemsa Banding: The chromosome ideogram comprised of 272 bands and the distribution of the bands among the 38 chromosomes are given in Table 1 & Figure 4**.** Out of 38 autosomes 14 chromosomes contained 9 bands, 13

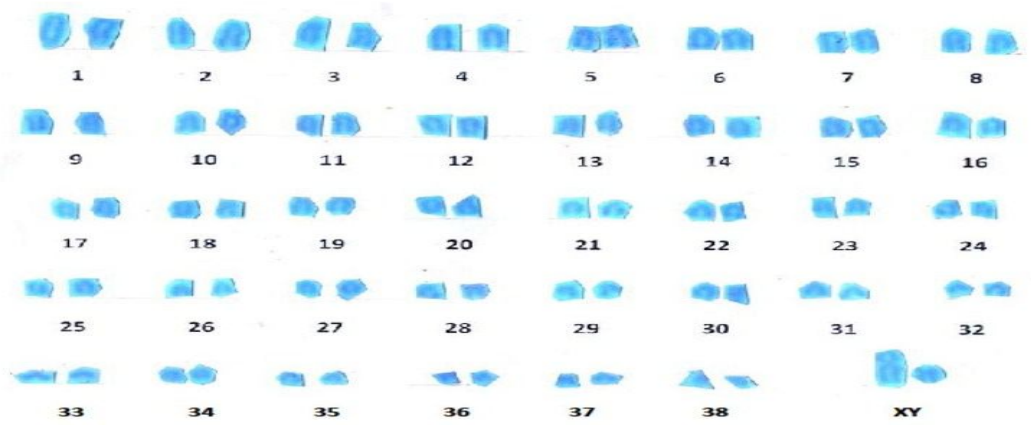

Figure 2: Karyology of Rajapalayam dog arranged in the decending order of chromosome size.

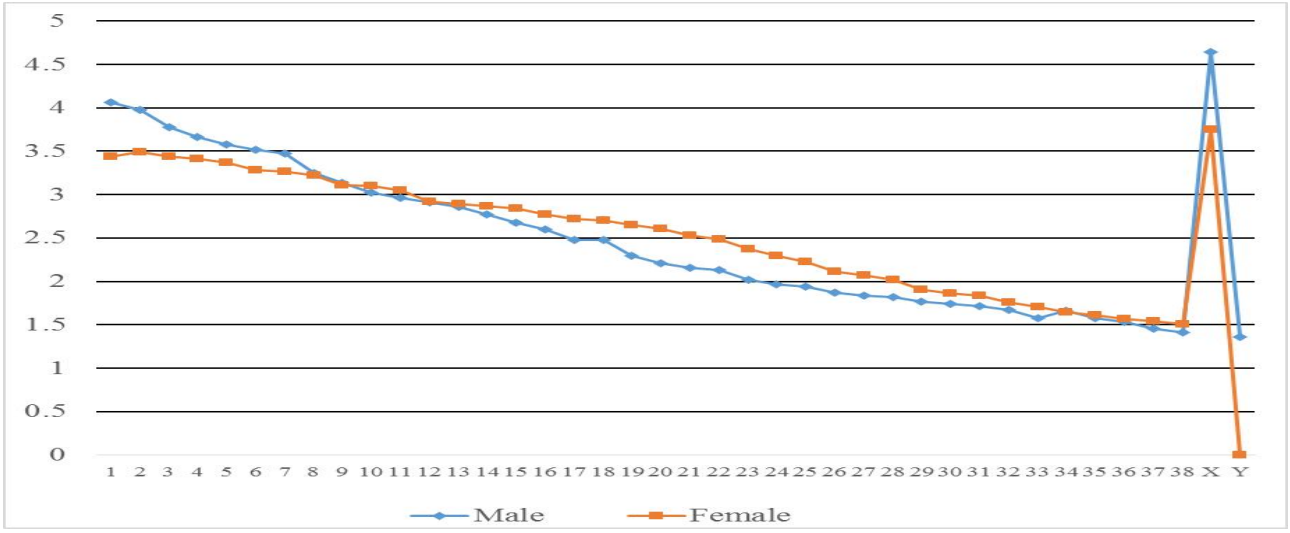

Figure 3: Graph showing the decline in the length of chromosome from 1 to 38

Table 1: Total number of bands observed through G- banding in Rajapalayam dog breed.

S.No.	No. of chromosomes	No. of bands	Total number of bands
1	01	15	15
2	01	13	13
3	02	11	22
4	14	09	126
5	13	07	091
6	01	06	006
7	06	05	030
Total	38	66	272

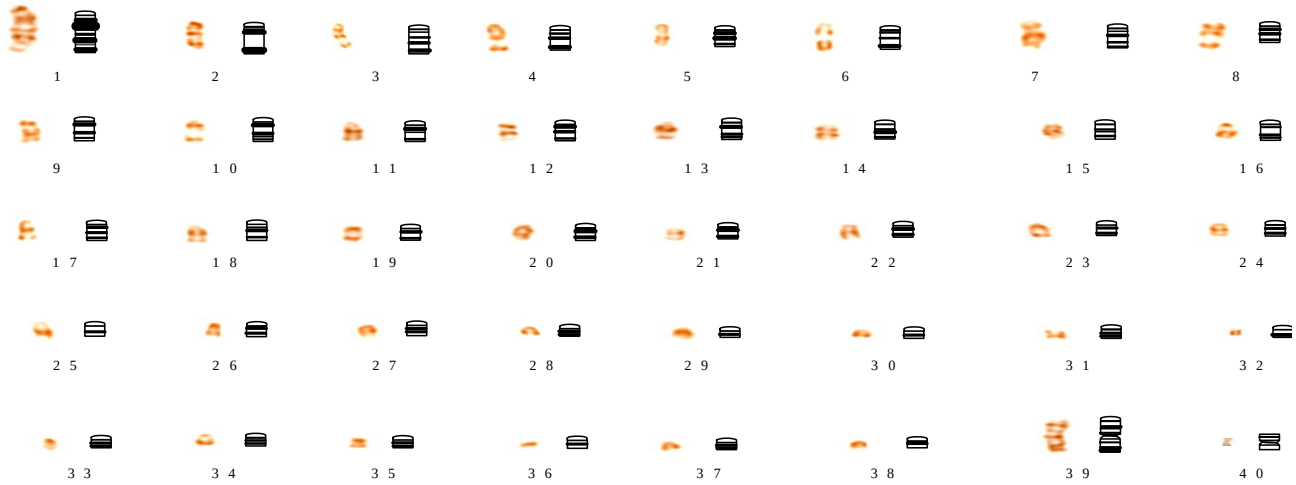

Figure 4: G-banding analysis of autosomes and sex chromosome of Rajapalayam dog

chromosomes contained 7 bands, 6 chromosomes contained 5 bands, 2 chromosomes contained 11 bands and one chromosome each showed 15, 13 and 6 bands, whereas the X and Y chromosomes contained 17 and 8 bands respectively through G banding analysis. But the karyotype of Chinese Raccoon dog (2n = 54 + B) reported to be composed of five pairs of bi-armed autosomes and 21 pairs of acrocentric autosomes, two bi-armed sex chromosomes and in addition, a variable (1 to 4) number of B chromosomes, with a total of 231 bands in A set of chromosomes (Ma¨kinen, 1986). The G banding of Chinese dog also revealed medium sized metacentric X chromosome and smallest subtelocentric Y chromosome and reported to have three types of B i.e., BI, BII and BIII chromosomes which resembles the Japanese Raccoon dogs (Pieñkowska, 2002). The banding pattern observed in the study enables for precise identification of the chromosomes. The mean centromeric index and the mean arm ratio (first report on Indian dogs) of Rajapalaym dog was observed to be 41.55 ± 0.40, 41.43 ± 1.37 and 1.41 ± 0.02 and 1.43 ± 0.08 for X and Y chromosomes, respectively (Table 2). Arm ratio for both X and Y chromosomes was observed to be more than 1, indicating that both the sex chromosomes were biarmed with submetacentric centromere. Until now, standard karyotype are available only for exotic dog breeds. The chromosome analysis of Rajapalayam dog breed of southern India revealed that the cytogenetic profile is different from that of Chinese Raccoon and Japanese Raccoon dogs which belongs to Canidae family with a basic chromosome number of 78 without B chromosome. The X chromosomes are longer in bitches than that of in dogs. G-banding analysis revealed higher number of bands distributed among the 38 chromosome in Indian dog breed as compared

Table 2: Centromeric index for X and Y chromosome in Rajapalayam dog

Chromosome	Short arm (S) mm	Long arm (L) mm = T mm	Total length (L + S)	Arm ratio L/S	Centromeric index (S/T) × 100
X	3.80	5.30	9.10	1.39	41.76
X	3.90	5.30	9.20	1.36	42.39
X	4.00	5.10	9.10	1.28	43.96
X	3.60	5.30	8.90	1.47	40.45
X	3.70	5.50	9.20	1.49	40.22
X	4.60	6.70	11.30	1.46	40.71
X	4.80	6.50	11.30	1.35	42.48
X	4.70	6.60	11.30	1.40	41.59
X	4.80	6.60	11.40	1.38	42.11
X	4.50	6.80	11.30	1.51	39.82
Mean ± SE	4.24 ± 0.15	5.97 ± 0.23	10.21 ± 0.37	1.41 ± 0.02	41.55 ± 0.40
Y	1.10	1.70	2.80	1.55	39.29
Y	1.20	1.50	2.70	1.25	44.44
Y	1.00	1.60	2.60	1.60	38.46
Y	1.20	1.50	2.70	1.26	44.44
Y	1.20	1.50	2.70	1.25	44.44
Y	0.90	1.50	2.40	1.67	37.50
Mean ± SE	1.10 ± 0.05	1.55 ± 0.03	2.65 ± 0.06	1.43 ± 0.08	41.43 ± 1.37

to that Chinese raccoon dog. Cytogentic characterization of Rajapalayam dog may form the basis for molecular genetic characterization and physical gene mapping through advanced techniques. The results of the present study will also give an insight knowledge to the researchers, dog breeders and kennel clubs about the karyology of Indian dog breeds and to detect any chromosomal abnormalities which may leads to fertility, growth and phenotypic abnormality related problems in this dog breed.

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REFERENCES

Barch M J, Lawee H J and Arsham M S. (1991). Peripheral blood culture. In: The ACT cytogenetics Laboratory Manual, [Barch, M.J. (Ed.)] 2nd edition Newyork, Raven Press. pp. 17-30.

Ma¨kinen Auli, Majra-Terttu Kuokkanen and Maija Valtonen. (1986). A chromosome-banding study in the Finnish and the Japanese Raccoon dog. *Hereditas* **10**: 97-105.

Moorhead P S, Nowell P C, Mellman WJ , Battips D M and Hungerford D.A. (1960). Chromosome preparation of leukocytes cultured from human peripheral blood. *Exp Cell Res* **20**: 613-616.

Pieñkowska A, Szczerbal I, Mäkinen A and Œwitoñski, M. (2002). G/Q-banded chromosome nomenclature of the Chinese Raccoon dog, *Nyctereutes procyonoides procyonoides* Gray. *Hereditas* **137**: 75-78.

Sumner A T, Evans H Tand Buckland R A. (1971). New technique for distinguishing between human chromosomes. *Nature N B* **232**: 31-32.

Suryawanshi A R, Kanadkhedkar H L and Umrikar U D. (2004). Chromosome analysis of Domestic Dogs. *Indian Vet J* **81**: 1071-1073.

Switonski M, Rogalska-Niznik N, Szczerbal I and Baer M. (2003). Chromosome polymorphism and karyotype evolution of four canids: the dog, red fox, arctic fox and raccoon dog. *Caryologia*. **56** (4): 375-385.

Topashka-Ancheva M, Gerasimova Ts, Dinchev V and Dimitrov K. (2009). Karyological data about the Bulgarian native dog breed “Karakachan Dog”. *Biotechnol Biotec Eq* **23**: sup1, 136-139, DOI: 10.1080/13102818.2009.10818384.

Wada M Y and Imai H T. (1991). On the Robertsonian polymorphism found in the Japanese Raccoon dog (*Nyctereutes procyonoides viverrinus*). *Jpn J Genet* **66**: 1–11.

Wada M Y, Suzuki T and Tsuchiya K. (1998). Re-examination of the chromosome homology between two subspecies of Japanese Raccoon dogs (*Nyctereutes procyonoides albus and N.p. viverrinus*). *Caryologia* **51** (1): 13-18.