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Cytogenetic characterization of Malaimadu cattle

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Abstract
Twenty Malaimadu cattle from its breeding tract were utilized to study the chromosome profile through short-term lymphocyte culture method. The diploid chromosome number was 60. All the 29 pairs of autosome were acrocentric, while X- was sub-metacentric and Y was acrocentric. The mean relative length of autosomes ranged from 1.76 to 5.44. The relative contribution of X and Y-chromosome was 5.12 ± 0.26 and 1.76 ± 0.08. The arm ratio and centromeric index of X-chromosome were 1.77 and 0.36 respectively. The chromosome banding showed C-positive dark band heterochromatin in all the acrocentric autosome. The present study revealed that the chromosome architecture of Malaimadu cattle was similar to that of the other registered breeds of indigenous cattle.

Keywords: Diploid, autosomes, heterochromatin, lymphocyte culture, acrocentric, sub-metacentric

Introduction
Malaimadu (locally called as Hill cattle) cattle were extensively reared in the adjoining Western Ghats areas of Madurai, Theni, Virudhunagar, Tirunelveli and Dindigul districts of Tamil Nadu. This cattle is also called as *Thozu madu* or *Vidi madu* due to the fact that it is reared in open type of housing without tethering. It has unique physical and functional characteristics such as medium size, slender, compact and fit to climb up the hills during the summer months, when the feed is scarce in the plains (Vivekanandan and Alagumalai 2013) [11].This genetic group is known for its draftability or endurance and also farmers make money by penning, manuring agricultural field and selling the male calves for sport events like jallikattu and reckla races. This population possesses high number of allelic variability with few alleles at low frequencies and was non-bottlenecked proving no recent reduction in effective population size. Moreover, Malaimadu cattle possess high within-breed genetic diversity with many polymorphic loci and hence could be recognised as a separate breed (Karthickeyan *et al*., 2019) [3]. Investigation of chromosomal profile in livestock provides a useful tool to identify the animals with congenital and acquired chromosome abnormalities whose elimination will help to maintain the herds cytogenetically clean and also in judging the fertility status of the animal even at a young age. Persual of available literature indicates that there is hardly any report on cytogenetic studies on Malaimadu cattle. Hence, the present study was conducted to unveil the chromosome architecture of this genetic group.

Materials and Methods
The present cytogenetic study was carried out on 10 males and 10 females of Malaimadu cattle from different villages of Theni, Virudhunagar and Dindigul districts of Tamil Nadu. Peripheral blood samples (2 to 3ml per animal) were collected aseptically from external jugular vein into a standard 10ml vaccutainer tube containing sodium heparin 68 USP as an anticoagulant. Immediately after collection, the samples were transported to the laboratory in ice and the culture was set within 16 hours of collection. The short term lymphocyte culture method, as described by Moorehead *et al* . (1960) [6] with slight modifications suitable to lab conditions was followed. The cocktail mixture consisting of 8ml of RPMI 1640 medium (GIBCO, USA), Benzathine pencillin (16µl), Streptomycin sulphate(10µl), 1.5ml of Foetal calf serum, 0.1 ml of Phytohaemagglutin -M, Heparin (7.5IU/ml of culture media) with blood sample of 0.5 ml was incubated for 72 hrs at 37°C and 8µg of colchicine was added one and half hour prior to harvesting the cells to arrest cell division at metaphase stage, followed by hypotonic treatment (0.075 M KCL) for 30 minutes at 37°C and fixation in Methanol: Glacial acetic acid (3:1). Approximately 20µl of the homogenous cell suspension was dropped on the chilled wet slides held at 45° angle from height of 2-3 feet with pressure. Slides were air dried and stained with 4% Giemsa stain for 20 minutes.

Slides were screened under 10x and 40x and 100x magnifications on a binocular phase contrast microscope (Olympus) and the best metaphase spreads were identified and captured with the digital camera.

The measurements of chromosomes for identification and pairing of homologous chromosomes were done using Ikaraos Karyotyping software module. According to the size, length and structure of chromosome, the karyotype was constructed. By the parameters for cytogenetic analysis, such as relative length, centromeric index, arm ratio and the size of chromosome, the proportionate share of different types of chromosome which contributed to the total genome were calculated.

The slides with good metaphase spreads were selected, aged at room temperature for 10 days and were subjected to C-banding studies as per the technique of Sumner (1972)[9] with slight modification. Slides were immersed in a coplin jar containing one per cent Barium hydroxide solution at 50ºC for 20 minutes. Then the slides were washed in distilled water and immersed in 2X SSC solutions for one hour at 60ºC. The slides were finally washed in triple glass distilled water and counterstained with 4 per cent Giemsa in phosphate buffer (pH 6.8) for 90 minutes. The slide was then observed under microscope and metaphase spread with good band pattern was selected and photographed.

Results and Discussion

A total number of 500 metaphases were examined phase contrast microscope and found that all metaphases contained modal diploid chromosome number (2n) of 60 with XY complement in male and XX complement in females which was in agreement with the findings of Kumarasamy *et al* . (2008) [4] in Umblachery, Faske *et al* . (2009) [2] in Dangi, Longkumer *et al* . (2015) [5] in Tho-Tho, Suresh *et al* . (2015) [10] in Malnad Gidda, Patel *et al* . (2016) [8] in Punganur and Parameswari *et al* . (2019) [7] in Alambadi breed of cattle.

The photographs showing the Giemsa stained mitotic metaphase chromosome spreads and karyotypes prepared for male and female Malaimadu cattle are shown in Figure 1 and 2.

The karyotype results showed that all the 29 pairs of autosomes of Malaimadu cattle were acrocentric. The X-chromosome was sub-metacentric and Y-chromosome was small acrocentric in morphology suggesting that the breed belongs to *Bos indicus*.

The relative length of the autosomes varied from 1.76 to 5.44 per cent. First chromosome was the longest acrocentric pair with a relative length of 5.44, X-chromosome was second longest and Y-chromosome was smallest of the chromosome complement with the relative length of 5.12 and 1.76 per cent respectively (Table 1). The ideogram was prepared based on the per cent relative length of the chromosomes, which reveals that there was more or less gradual rate of reduction of relative length from 1st pair to 29th pair of autosomes (Figure 4). The mean arm ratio, and centromeric index of X-chromosome are presented in Table 2 and Figure 5,6. Arm ratio was 1.77 which is slightly lower than the reports of Kumarasamy *et al* . (2008) [4], Suresh *et al* . (2015) [10] and Parameswari *et al* . (2019) [7], whereas, lower values of 1.55 was reported by Bharathi *et al* . (2015) [1] in Punganur cattle. Further, the centromeric index of X-chromosome was 0.36, which confirmed its sub-metacentric nature and this was similar to reports of Kumarasamy *et al* . (2008) [4], Suresh *et al* . (2015) [10] and Parameswari *et al* . (2019) [7].

The C-banded metaphase chromosomal spreads exhibited a distinct dark stain of heterochromatin at the centromeric region of all the autosomes, where as the X- and Y-chromosomes were uniformly stained without banding pattern (Figure 3). The density of the centromeric stain appears to be more in the larger chromosome and it reduces as the size of the acrocentric chromosome decreases.

Table 1: Cytogenetic parameters of Malaimadu cattle

Chromosome no. (Pair)	Relative length		Overall Relative length	Chromosome type	C – Band (+/-)
	Male	Female			
1	5.43 ± 0.07	5.45 ± 0.09	5.44 ± 0.01	a	+
2	4.86 ± 0.08	4.76 ± 0.11	4.81 ± 0.05	a	+
3	4.49 ± 0.06	4.47 ± 0.07	4.48 ± 0.01	a	+
4	4.33 ± 0.04	4.30 ± 0.05	4.32 ± 0.02	a	+
5	4.20± 0.05	4.14 ± 0.07	4.17 ± 0.03	a	+
6	4.11 ± 0.05	4.05 ± 0.07	4.08 ± 0.03	a	+
7	4.03 ± 0.07	3.98 ± 0.05	4.01 ± 0.03	a	+
8	3.96 ± 0.06	3.85 ± 0.05	3.91 ± 0.05	a	+
9	3.80 ± 0.02	3.79 ± 0.05	3.80 ± 0.00	a	+
10	3.58 ± 0.04	3.73 ± 0.03	3.66 ± 0.07	a	+
11	3.43 ± 0.04	3.59 ± 0.04	3.51 ± 0.08	a	+
12	3.30 ± 0.04	3.45 ± 0.02	3.38 ± 0.08	a	+
13	3.25 ± 0.06	3.34 ± 0.02	3.30 ± 0.04	a	+
14	3.07 ± 0.02	3.25 ± 0.04	3.16 ± 0.09	a	+
15	3.01 ± 0.01	3.19 ± 0.02	3.10 ± 0.09	a	+
16	2.96 ± 0.01	3.10 ± 0.04	3.03 ± 0.07	a	+
17	2.90 ± 0.01	2.99 ± 0.04	2.95 ± 0.05	a	+
18	2.80 ± 0.00	2.90 ± 0.03	2.85 ± 0.05	a	+
19	2.75 ± 0.01	2.82 ± 0.04	2.79 ± 0.03	a	+
20	2.67 ± 0.02	2.70 ± 0.06	2.69 ± 0.02	a	+
21	2.60 ± 0.01	2.63 ± 0.04	2.62 ± 0.01	a	+
22	2.53 ± 0.01	2.59 ± 0.05	2.56 ± 0.03	a	+
23	2.45 ± 0.00	2.53 ± 0.04	2.49 ± 0.04	a	+
24	2.40 ± 0.02	2.46 ± 0.04	2.43 ± 0.03	a	+
25	2.30 ± 0.05	2.39 ± 0.05	2.35 ± 0.05	a	+

26	2.19 ± 0.02	2.28 ± 0.06	2.24 ± 0.04	a	+
27	2.14 ± 0.04	2.15 ± 0.04	2.15 ± 0.00	a	+
28	1.94 ± 0.07	1.99 ± 0.05	1.97 ± 0.03	a	+
29	1.77 ± 0.04	1.75 ± 0.06	1.76 ±0.01	a	+
X	4.86 ±0.14	5.38 ± 0.16	5.12 ± 0.26	Sm	-
Y	1.76 ± 0.08	-	-	a	-

a: acrocentric; Sm: sub-metacentric; +: present; -: absent

Table 2: Mean arm ratio and Centromeric index (expressed in percentage) of chromosomes of Malaimadu cattle

Parameters	Male	Female	Overall
Arm ratio	1.74 ± 0.07	1.79 ± 0.03	1.77 ± 0.03
Centromeric index	0.36 ± 0.01	0.359 ± 0.00	0.36 ± 0.00

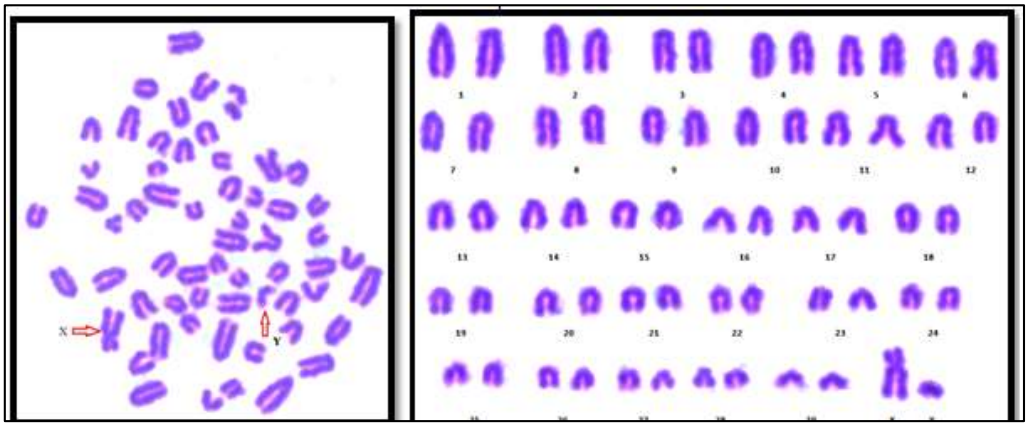

Fig 1: Metaphase spread and karyotype of Malaimadu male cattle (60, XY)

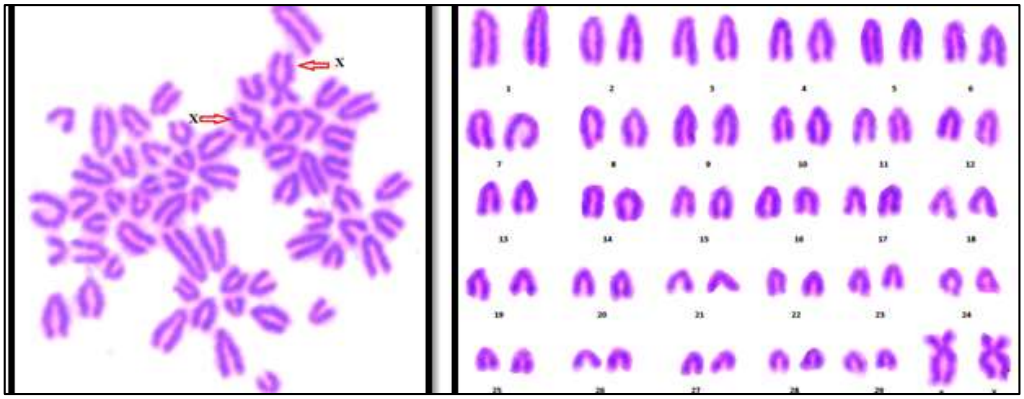

Fig 2: Metaphase spread and karyotype of Malaimadu female cattle (60, XX)

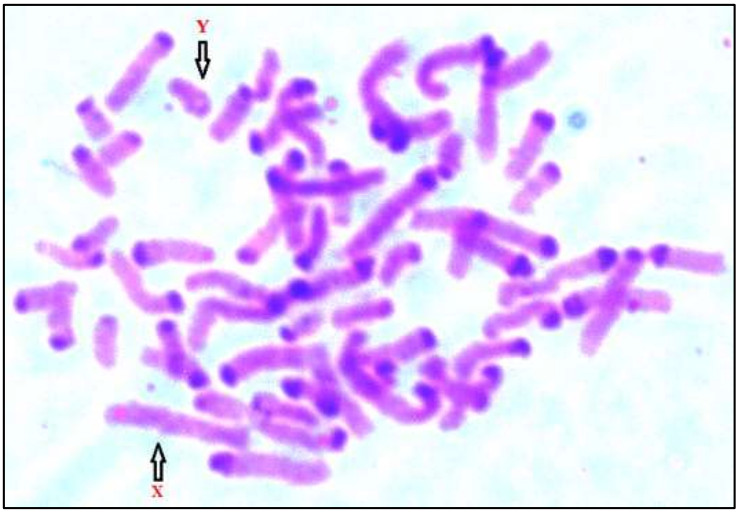

Fig 3: C-banded metaphase chromosomes of *Malai Madu* male cattle (60, XY)

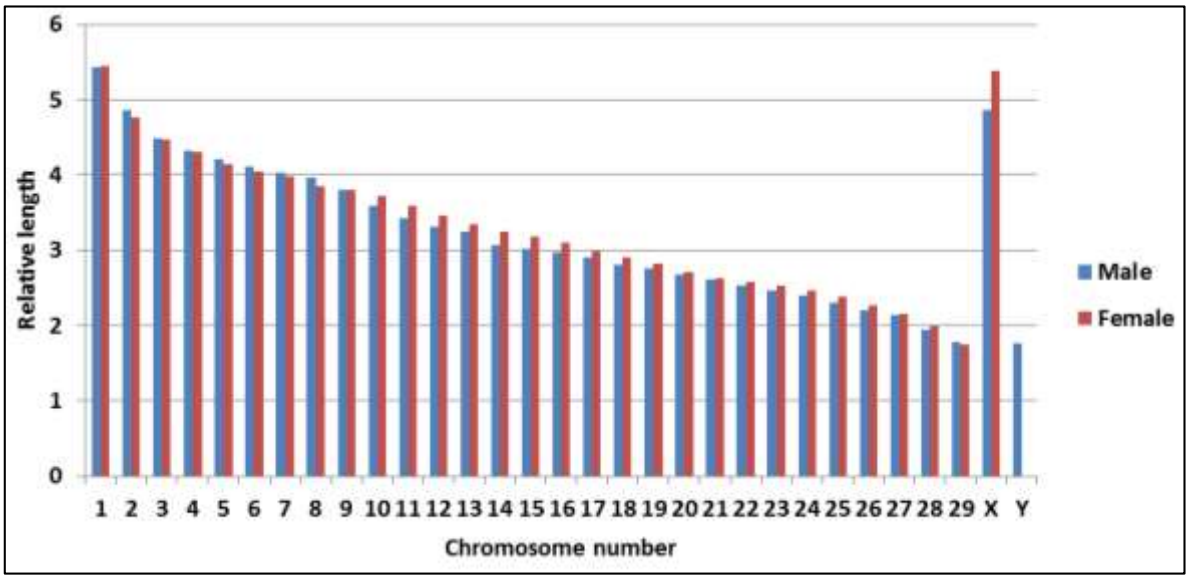

Fig 4: Ideogram representing the relative length of chromosomes of Malaimadu cattle

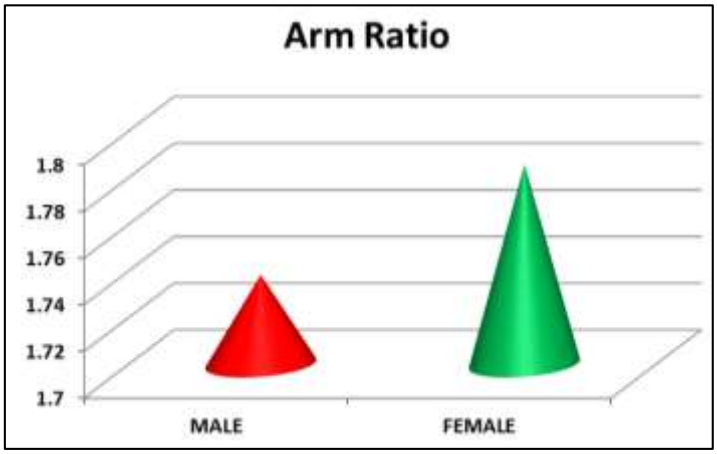

Fig 5: Mean arm ratio (expressed in percentage) of chromosomes of Malaimadu cattle

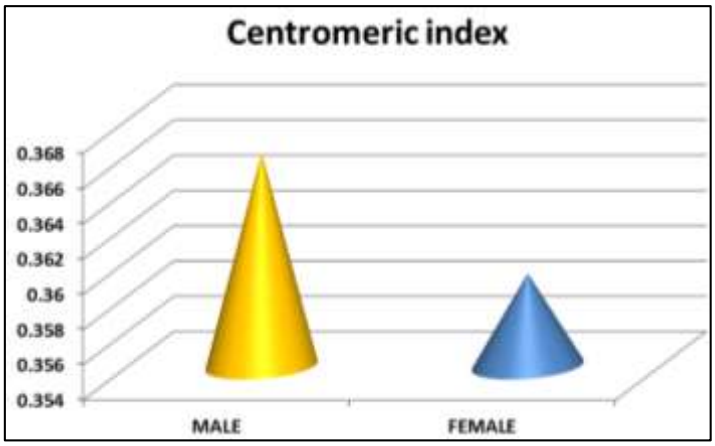

Fig 6: Centromeric index (expressed in percentage) of chromosomes of Malaimadu cattle

Conclusion

The present study found that the cytogenetic parameters of the Malaimadu cattle are in agreement with the other recognized native breeds of *Bos indicus*, thus confirming that karyotype profile of investigated animals do not differ from standard karyotype of bovine species. However, the present study will be a platform for further comparative studies with other breeds of same species by molecular cytogenetics and molecular techniques.

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