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Original Research

Cytogenetic Screening of Breeding Herd of Malnad Gidda Cattle

R. Jayashree[1], B. H. Rudresh[2], G. M. Sateesha[3], Sudarshan Gadad[4], Vemula Harshini[5] and Vijaykumar B. Shettar[6]

[1] Associate Professor and Head, Department of Animal Genetics and Breeding, Veterinary College, Shimoga, KVAFSU, Karnataka, INDIA
[2] Assistant Professor, Dept. of Animal Genetics and Breeding, Veterinary College, Shimoga, KVAFSU, Karnataka, INDIA
[3] Assistant Professor, Dept. of Livestock farm complex, Veterinary College, Shimoga KVAFSU, Karnataka, INDIA
[4] Assistant Professor, Dept. of Animal Genetics and Breeding, Veterinary College, Shimoga, KVAFSU, Karnataka, INDIA
[5]PhD Scholar, Dept. of AGB, Madras Veterinary College, Chennai TANUVAS, Tamil Nadu, INDIA
[6]Professor Head, Dept. of AGB, Veterinary College, Gadag, KVAFSU, Karnataka, INDIA

*Corresponding Author: jayavet@gmail.com

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Abstract

Breeding herd of Malnad Gidda cattle (12 No.s) from the Malnad Gidda Research and Information Centre, Veterinary College, Shimoga were screened for the chromosome profile through short-term lymphocyte culture method. The methodology adopted for invitro lymphocyte proliferation was standardized and the proliferation was to the extent of 72.27 per cent, 23.34 per cent of the proliferated cells have reached metaphase stage and in that 4.39 per cent of the chromosome spreads were fit for karyotyping. This revealed that the procedure adopted was suitable for karyotyping of Malnad Gidda breed of cattle. The diploid chromosome number was 60. All the 29 pairs of autosomes were acrocentric, while X- was sub-metacentric and Y was acrocentric. The mean relative length of autosomes ranged from 5.214 ±0.061 to 2.230 ±0.074, The X chromosome was the largest in the karyotype5.719 ±0.177 while the Y chromosome was the smallest with a relative length of 1.060 ±0.504. Among the animals screened no chromosomal abnormalities was observed. The present study also revealed that the chromosome architecture of Malnad Gidda cattle was similar to that of other indigenous breeds of cattle of India.

Keywords: Arm Ratio, Autosomes, Chromosomes, Karyotype, Malnad Gidda Cattle

Introduction

Malnad Gidda is a small statured, multipurpose breed of cattle reared by the farmers in hilly terrain of Western Ghats of Karnataka. This breed is known for its inherent strength to withstand adverse climatic conditions and are well adapted to hot humid climate of Malnad region. They are generally left for grazing in the open fields or they go uphill to the fringes of the forest and have the habit of grazing various kinds of weeds as reported by Ashok (2000). Farmers in this region mostly adopt organic farming practices for their cash crops like areca, pepper and vanilla for which they need plenty of manure from cow dung. These animals of about 20 to 25 in numbers are enclosed in sheds with a bedding of tree leaves during the night so that the dung voided is collected in the floor along with the leaves. Daily fresh layer of tree leaves is spread over the flooring and after a few days they are removed and composted, by this way, the bulk of the composted manure is increased. Hence, there is great demand for the cow dung manure obtained from this Malnad Gidda breed of cattle.

A pure breeding herd of Malnad Gidda breed of cattle are maintained exclusively in Malnad Gidda Research and Information Centre at Veterinary College, Shimoga. These farm serves as a model unit for training and demonstration to farmers and also for performance recording. It is always essential to perform cytogenetic screening to any breeding herd so that we shall safely guard the future generations from any gross chromosomal anomalies. Chromosomal aberrations might be transmitted or spontaneous generated during mitotic or meiotic cell divisions. Therefore, complete eradication of chromosomal aberrations and the regular chromosomal screening especially of breeding bulls at the early age ought to be done. Chromosomal aberrations have influence on the phenotypic expression of the reproduction and fertility traits and affects the selection program as reported by Slavica *et al* (2006), Raudsepp and Chowdhary (2016) and Jamir *et al.* (2015). However, the incidences of structural and numerical chromosomal abnormalities are very less in *Bos indicus* breeds of India as reported elsewhere. The standardization of the techniques of studies on cattle chromosomes were studied by Popescu *et al* (1996) which were later modified with suitable culture media and mitogens. In *Bos indicus* cattle chromosomes the autosomes are usually acrocentric and X chromosome is submetacentric and Y chromosome was smallest acrocentric, Halnan *et al* (1981). Cytogenetic screening was purposefully done to the Malnad Gidda breeding herd in Veterinary College, Shimoga to document the chromosomal profile of the animals and to standardize the technique of invitro lymphocyte cell culture in the existing laboratory conditions by calculating the mitotic drive and mitotic index. Mitotic drive indicates the percentage of lymphocytes proliferated and the mitotic index indicates the percentage of proliferated lymphocytes to have reached the metaphase stage. The study also included various cytogenetic parameters such as relative length, centromeric index and arm ratio.

Materials and Methods

A total of 12 animals (7 females and 5 males) maintained in the Malnad Gidda research and information Centre at Veterinary College, Shimoga were screened in this study. About 5 ml of blood from the external jugular vein was drawn in sterile heparinized vacutainer tubes. The short-term lymphocyte culture method Moorehead *et al.* (1960) was followed. The culture was set using the chromosome complete medium (Euroclone- EK AMTB-100). Freshly collected whole blood (0.5 ml) was added aseptically in the laminar air flow to the 7 ml of complete medium and incubated at 37ºC at 5% CO2 concentration for 72 hrs. Colchicine (0.8µg) was added to each culture tube, 90 minutes prior to harvesting the culture. After 72 hrs culture tubes were centrifuged at 1500 rpm for 10 minutes and discarded the supernatant fluid. The cell pellet was treated with 7ml of 0.075M KCL at 37ºC for 30 minutes, centrifuged for 10 minutes at 1500 rpm and discarded the supernatant fluid. Freshly prepared and chilled (-20ºC) Carnoy's fixative solution (3:1 ratio of Methanol: Glacial acetic acid) was added to the cell pellet, and the contents were mixed well. Then this was centrifuged at 1000 rpm and the supernatant fluid was discarded. The washings with fixative solution were repeated several times until a white pellet appears. Approximately 20µl cell suspension was dropped onto the chilled wet slides held at 45º angle from two feet height. Air dried slides were stained with 4% Giemsa for 20 minutes. Slides were examined under binocular microscope and good metaphase spreads were photographed. 50 good chromosomal spreads of the male and female were printed and the lengths of the individual chromosomes was measured manually using Vernier calipers. All the measured data were analyzed using Microsoft Excel. The relative length percentage of the chromosomes was calculated as the length of the individual chromosome over the total length of all the autosomes and multiplied by 100. Mitotic drive was calculated as the percentage of total lymphocytes that were in metaphase, Rathnasabapathy and Ganesh (1980). Differential count from the slides prepared for all samples were made, to work out the mitotic drive and mitotic index.

The arm ratio was calculated as ratio of the average length of the long arm over the short arm for the X chromosomes of the female and male separately (L/S) as reported by Levan *et al* (1964). Its values can range from 1 (if S = L) to +∞ (the limit for S = 0). The centromeric index was calculated as the proportion of average length of the short arm with the whole chromosome for the S/(L+S). Its values can range from 0.5 (if S = L) to 0 (if S = 0), Huziwara (1962). The result obtained were tabulated and images of the spreads were documented.

Results

The diploid chromosome number was 60 in all of the samples which revealed no numerical chromosomal abnormalities in the breeding herd of Malnad Gidda cattle. All the 29 pairs of autosomes were acrocentric, while X- was sub-metacentric and Y was acrocentric (Plate 1 A & 1 B). Karyotyping was done using the Karyotyping software and is represented in Plate 2 A & 2 B. This revealed that there was no structural deviation and all the samples belonged to the *Bos indicus* group based on the Y chromosome morphology.

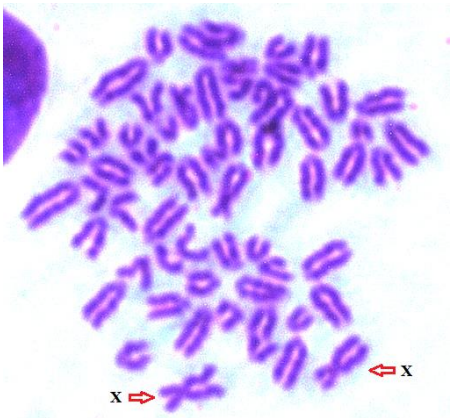

Plate1 A: Chromosome spread of female

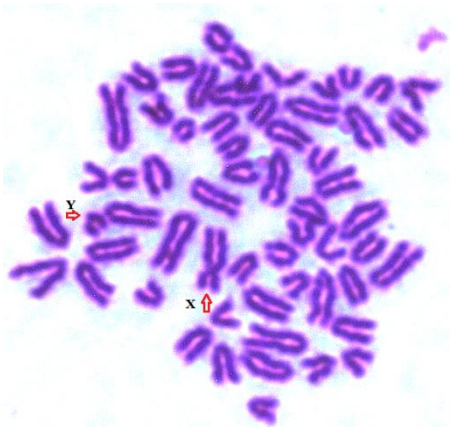

Plate 1 B: Chromosome spread of male

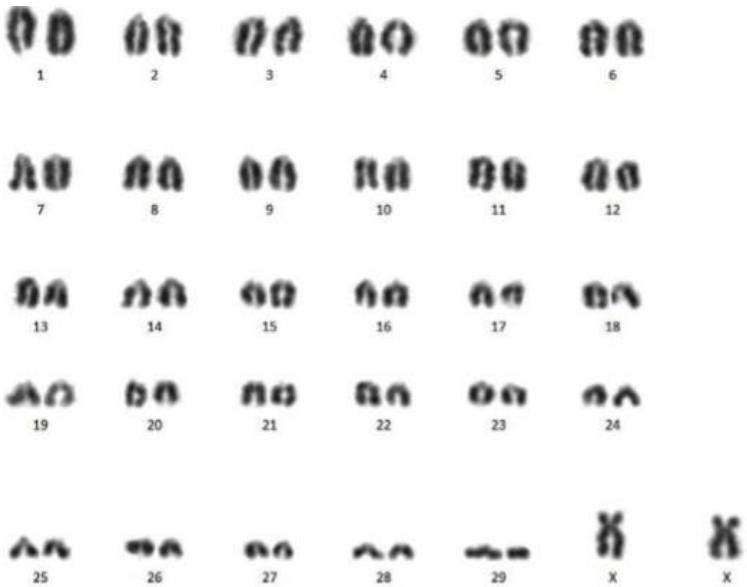

Plate 2 A: Karyotype of the Malnad Gidda female

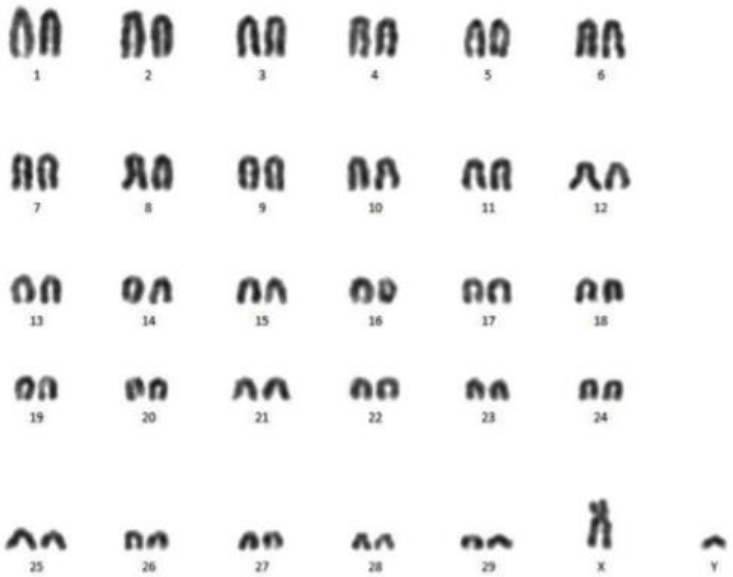

Plate 2 B: Karyotype of the Malnad Gidda male

The methodology adopted for invitro lymphocyte proliferation was standardized and the proliferation (Mitotic drive) was to the extent of 72.27 per cent, Mitotic index observed was 23.34 per cent of the proliferated cells have reached metaphase stage. Out of these 4.39 per cent of the chromosome spreads were fit for karyotyping. This revealed the suitability of the procedure adopted for in-vitro lymphocyte culture done in the existing laboratory conditions. The overall mean relative length of autosomes ranged from to 2.230 ± 0.074 to 5.214 ± 0.061, The relative length percent was in the range of 2.251 ± 0.118 to 5.153 ± 0.0831 and 2.209 ± 0.083 to 5.276 ± 0.085 for females and males respectively. The X chromosome was the largest in the karyotype 5.719 ± 0.177 while the Y chromosome was the smallest with a relative length of 1.060 ± 0.504 (Table 1 & Figure 1). The arm ratio in females and in males were calculated for the X chromosomes as 1.80 ± 0.004 and 2.236 ± 0.002 respectively. The Centromeric Index for female was calculated as 35.77 ± 0.0045 per cent and for male was 38.63 ± 0.0022 percent.

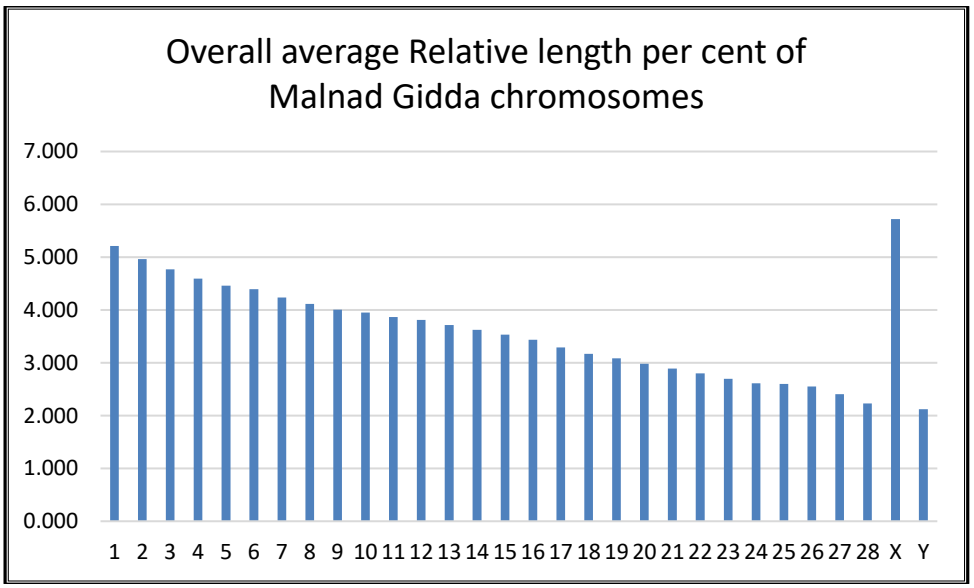

Figure 1: Relative length percentage of autosomes and X and Y chromosomes

Table 1: Relative length of chromosomes

Chromosome No.	Female	SE	Chromosome No.	Male	SE	Overall Average	SE
1	5.153	0.083	1	5.276	0.085	5.214	0.061
2	4.932	0.084	2	4.999	0.059	4.966	0.054
3	4.722	0.051	3	4.813	0.052	4.767	0.038
4	4.583	0.05	4	4.603	0.044	4.593	0.034
5	4.41	0.035	5	4.511	0.039	4.46	0.029
6	4.352	0.04	6	4.43	0.033	4.391	0.028
7	4.188	0.106	7	4.286	0.026	4.237	0.06
8	4.078	0.111	8	4.156	0.022	4.117	0.062
9	4.069	0.12	9	4.041	0.036	4.004	0.068
10	3.967	0.165	10	3.91	0.034	3.868	0.092
11	3.943	0.14	11	3.83	0.032	3.95	0.084
12	3.826	0.134	12	3.679	0.008	3.811	0.082
13	3.815	0.142	13	3.618	0.021	3.717	0.082
14	3.721	0.146	14	3.529	0.026	3.625	0.085
15	3.641	0.136	15	3.426	0.035	3.533	0.081
16	3.533	0.101	16	3.338	0.024	3.436	0.063
17	3.374	0.036	17	3.209	0.015	3.292	0.031
18	3.222	0.028	18	3.118	0.014	3.17	0.022
19	3.107	0.053	19	3.058	0.013	3.082	0.03
20	2.975	0.092	20	2.987	0.024	2.981	0.051
21	2.849	0.088	21	2.935	0.03	2.892	0.051
22	2.714	0.13	22	2.888	0.031	2.801	0.076
23	2.578	0.19	23	2.809	0.022	2.694	0.109
24	2.571	0.196	24	2.746	0.022	2.611	0.114
25	2.539	0.064	25	2.664	0.018	2.601	0.04
26	2.475	0.116	26	2.529	0.022	2.552	0.064
27	2.408	0.092	27	2.403	0.037	2.405	0.053
28	2.251	0.118	28	2.209	0.083	2.23	0.074
X	6.006	0.302	X	5.629	0.095	5.719	0.177
X	5.522	0.234	Y	2.12	0.099	1.06	0.504

Discussion

The normal karyotype of cattle (*Bos taurus* and *Bos indicus*) comprises of 2n=60 chromosomes of which fifty-eight are acrocentric autosomes and two sex-chromosomes, Sasaki and Makino (1962); Gustavsson (1969); Ahmad *et al* (2004). In this study on the Malnad gidda breed of cattle also the 2N was found to be 60 (58 autosomes and 2 sex chromosomes: XY). The simple cytological technique of invitro proliferation of lymphocytes has led to the discovery morphological differences of the Y chromosomes i.e. *Bos indicus* have an acrocentric whereas *Bos taurus* have metacentric Y chromosome. Malnadgidda breed of cattle belonging to the *Bos indicus* group was revealed by the acrocentric Y chromosomes in the breeding herd studied. The morphologic difference between the Y chromosomes of the two subspecies can be attributed to a pericentric inversion, Goldammer *et al.* (1997, Di Meo *et al.* (2005) and they concluded that a transposition of the centromere or a pericentric inversion occurred, which differentiated the Y chromosome of B. taurus from that of B. indicus. In this study all the autosomes were acrocentric and were in the decreasing order of length as found in other breeds of bovines reported by different authors. The X chromosome is the largest sub metacentric chromosome and the Y chromosome was the smallest acrocentric in the Karytype. These were in corroboration with the studies on other Indian breeds Deoni, Ongole,

Umbalachery and Tho tho cattle by various researchers, Balaji *et al.* (2006), Kumarasamy *et al.* (2006, 2008), Long kumer *et al.* (2015 and Bharti A *et al.* (2017).

The relative lengths of each chromosome were measured as the percentage of it to the total haploid genome length (excluding Y-chromosome). The overall mean relative length of autosomes ranged from to 2.230 ± 0.074 to 5.214 ± 0.061, in the breeding herd of Malnad Gidda breed that was studied. The relative length percent was in the range of 2.251 ± 0.118 to 5.153 ± 0.0831 and 2.209 ± 0.083 to 5.276 ± 0.085 for females and males respectively. This was in agreement with the earlier studies in Malnad Gidda cattle where in the relative length (RL) of autosomes progressively decreased from 4.85 ± 0.05 to 1.85 ± 0.03 per cent and from 4.95 ± 0.05 to 1.58 ± 0.03 per cent in male and female Malnad Gidda cattle respectively, Suresh *et al.* (2015).

The average relative length X chromosome 5.719 ± 0.177 while the Y chromosome was the smallest with a relative length of 1.060 ± 0.504. The contribution of X chromosome of female and male was 5.718 ± 0.210, 5.629 ± 0.095 per cent respectively and this was in agreement with the report of Suresh *et al*. (2015) in Malnad Gidda cattle wherein they reported 5.09 ± 0.05 and 5.15 ± 0.05 per cent in male and female Malnad Gidda, respectively. In Ongole cattle the mean relative length of autosomes ranged from 1.92 ± 0.01 to 5.24 ± 0.02. The X-chromosome was the largest in the karyotype (5.42 ± 0.03), while the Y was the smallest (1.79 ± 0.02), Balaji *et al.* (2006), Kumarasamy *et al.* (2008). In Tho tho cattle the mean relative length percent of the autosomal chromosomes varied from 5.48 ± 0.107 to 1.79 ± 0.105 in male and 5.31 ± 0.148 to 1.86 ± 0.055 in female, respectively, Longkumer *et al* (2015). Similar results were reported in other Bos indicus breeds like Punagnur, Umbalacherry, Ongole, Deoni and Hariana breed of cattle (Kumar *et al*., 2003; Rao, 1995; Nagpure *et al.,* 2001; Appannavar *et al*., 2004; Kumaraswamy *et al*., 2008). The arm ratio of the X chromosomes in females and males were calculated as 1.80 ± 0.004 and 2.236 ± 0.002 respectively. This was in agreement with that reported as 2.60 ± 0.09 and 2.23 ± 0.06 for male and female respectively in Tho-tho cattle breed, Longkumer *et al.* (2015). The Centromeric Index was 35.77 ± 0.0045 and 38.63 ± 0.0022 percent female and male respectively which was corroborating with the earlier studies in Malnad gidda where it was reported as 0.32 ± 0.01 respectively. Ahmad *et al*. (2020) in their studies on Karan Fries and Tharparkar males have also reported no structural or numerical chromosomal abnormalities in breeding males.

Conclusion

The present study revealed the normal status of the chromosome architecture of the breeding herd of Malnad Gidda breed of cattle in the Malnad Gidda research and information Centre. The technique adopted was suitable and shall be adopted for further screening of the animals in the breeding tract.

Conflict of Interests

There is no conflict of interest.

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