G-banded Chromosomal Pattern and DNA Extractions of Two Bovine Species, *Bos taurus* (Linnaeus, 1758) and *Bos frontalis* (Lambert, 1804)

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Abstract

Cytogenetic investigation was carried out on 14 bovine individuals (seven bulls and seven cows) from Pyinmabin Dairy Cattle Farm Livestock and Zoological Garden, Yangon. G-banded metaphase karyotypes in cattle (*Bos taurus* and *Bos frontalis*) were studied using the leucocyte culture method and G-banding technique. In addition, the genomic DNA extracted from the lymphocyte (5mL volume) were measured for purity and concentration. The normal chromosome complements of *Bos taurus* (n=10) were 2n = 60 and those of *Bos frontalis* (n=4) were 2n = 58. Autosomal pairs in both sexes of *Bos taurus* were acrocentric. The X chromosome was the largest submetacetric and smallest submetacentric of Y chromosome. *Bos frontalis* had a diploid chromosome number 2n = 58 made up of two submetacentric autosomes (different in size) and 54 acrocentric autosomes. The sex X chromosomes in females were a pair of submetacentric. The Y chromosome had a small submetacentric in males of two bovine species. The Y chromosome were found to be significantly different ($p < 0.05$). This study is a contribution to the construction of clearer standard G-banded karyotypes for this important bovine species.

Keywords: karyotypes, lymphocyte, banding, DNA extractions, bovine

INTRODUCTION

Cytogenetics is the study of the structure, number, size and shape of chromosome material. It includes routine analysis of G-banded chromosome and other cytogenetic banding techniques (Tjio and Levan, 1956).

Genes are located along the chromosome. A gene is the basic functional unit of heredity. Genes are a part of the chromosomes that resides in the nucleus of body cells (Court-Brown, 1969).

Gene composed of deoxyribonucleic acid (DNA), is the basic functional unit of heredity. In the cell, the greatest amount of DNA remains concentrated in the nucleus especially in the chromosome. The amount of DNA has been found to be constant in cells of each species (Verma and Agarwal, 1978).

The DNA found in the nucleus of all eukaryotic cells is a double stranded molecule. DNA can be extracted from virtually any blood sample. The quality and quantity of the DNA obtained will vary depending on the size, age and white blood cell count of the sample (Dacie and Lewis, 1995).

The eukaryotic chromosomes have been classified into autosomes and sex chromosomes. The autosomes exceed the sex chromosome in number. The nuclei of the somatic cells are said to contain the diploid number “2n” of chromosomes, one maternally and

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one paternally derived chromosome. The germ cells contain only half the number of chromosomes and are said to be haploid number "n" (Barr *et. al.*, 1962).

Giemsa banding (G-banding) has become the most widely-used technique for the routine staining of mammalian chromosome. The use of the G-banding techniques can identify not only the particular chromosomes involved in structural rearrangements but also the specific band numbers of the breakpoints with the chromosome (Iannuzzi, 1996).

Taking this into consideration, the research work is conducted on G-banded chromosomal pattern and DNA extraction of two bovine species.

The objectives of the present study are;

- to study the morphology of the chromosomes in the two bovine species
- to analyze the karyotypes of the two bovine species
- to observe the Giemsa banding pattern of the karyotypes in the two bovine species
- to extract deoxyribonucleic acid (DNA) from the peripheral blood of the two bovine species

MATERIALS AND METHODS

Study site

Blood samples were collected from Pyinmabin dairy cattle farm livestock, Mingaladon Township and Yangon Zoological Gardens, Mingalartaungnyunt Township in Yangon Region (Fig 1).

Lymphocyte culture, karyotyping and Giemsa banding of chromosomes were conducted in the Laboratory of Pathology Research Division, Department of Medical Research (DMR) in Yangon.

Extraction of DNA from the lymphocytes were analysed for purity and concentration in the Department of National Health Laboratory (NHL), Yangon.

Fig 1. Map of study site showing Pyinmabin Dairy Farm and Yangon Zoological Garden (Source: Google Earth, 2020)

Study period

The study period lasted from October 2017 to October 2019.

Blood samples collection

Blood samples (3mL/cattle) were collected from the jugular veins of the studied species, by disposable plastic syringes (5mL) and put into sterilized glass bottles with rubbers stoppers and heparinized. The blood samples were brought to the DMR.

Five domestic cattles and two mithun cattles (both sexes) were respectively selected for blood sample collection (Plate 1).

Method

Peripheral blood was cultured and then colchicine was added for mitotic arrest. Harvesting was done by treatment with hyptonic solution, fixed with glacial acetic acid and methanol (1:3). This destroyed the red blood cells and made the lymphocytes easier to rupture. A drop of the lymphocyte suspension cells was placed on heated clean glass slides. The cells left on the slide were dried and then the slides were incubated overnight (16-18h) at 56-60^{o}C for optimal results. After overnight incubation, the slides were placed 20-40 sec in the 0.0005% Trypsin (Difco) solution in a Coplinjar. Slides were then thoroughly with 70 mL of cold (refrigerated) Phosphate-buffered saline (PBS) (pH 7.0). The slides were stained for 10 min in 2.5 mL of Gimesa (Gurr's) solution and rinsed the slides in distilled watered and air-dried.

The preparations were examined under a light microscope with 100x magnification and the mitotic plates with clear and well-distributed chromosomes were photographed. Chromosomes were classified according to Reading Conference (1980) standard (Plate 2).

For extraction of DNA

A volume of 5mL blood sample was frozen at – 20^{o}C in EDTA for each tube. The tubes were thawed and centrifuged at 2500 rpm for 15 minutes, it was discarded and added with 2mL of phosphate-puffered saline (PBS) + 10% Nonidet P-40 (NP40). The sample was homogenized by vortexing each tube. The tubes were then added with 0.5mL of lysis solution and 10% SDS was added and the mixture was incubated at 37^{o}C for 15 min. An equal volume of chloroform isoamyl alcohol (24%) and 5mL of phenol were also added to the tubes and then shaken very gently by autovorter mixes for 5 min. The upper aqueous phase was transferred to a new tube. Each new tube was added with 2.5 volumes of absolute ethanol. The DNA was precipitated as a "cotton wool" by the addition of 1mL of 70% ethanol and centrifuged in a microcentrifuge at 13000 rpm for 5 min. DNA pellet was the suspended addition with 50 μL of TE depending on the size of the pellet. The DNA solution microcentrifuge tubes were incubated at 37^{o}C overnight until DNA was dissolved completely. DNA could be stored for long periods at 4^{o}C of frozen at -20^{o}C (Plate 3).

Determining the DNA Concentration

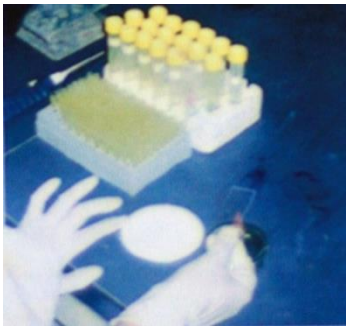
5μL of the DNA solution were taken and diluted into 245 μL of water. Then, the DNA solution was mixed well by vortexing and read the absorbance (A) in a spectrophotometer at 260 nm against a water DNA at a concentration of 50 μL/mL. Therefore, multiply the A reading obtained by 2500 to get the concentration of the original DNA solution in μg/mL. The ratio of the A_{260} to the A_{280} gives an indication as to the purity of the DNA solution. This ration should be in the range 1.7 – 2.0 (Vulliamy and Kaeda, 1995) (Plate 3).

Statistical analysis

The relationship between the chromosome mean total length and the number of chromosome pairs of the species was tested by the F Statistic Test (F-test) method.

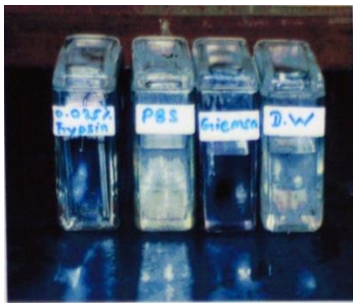
Plate 1. Blood sample collection

Reagent



Cuture medium

Incubated at 60^{o}C for overnight



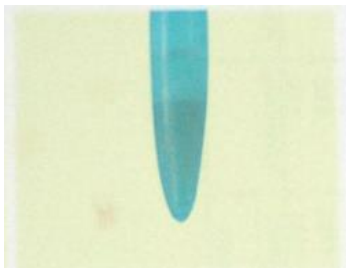
Trypsin solution

Giemsa solution

Light microscope

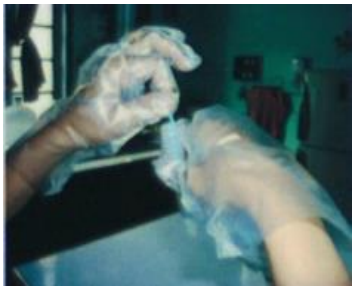
Plate 2. Procedure of G-banding

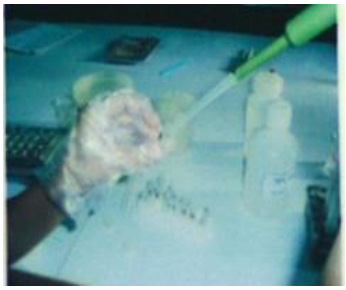
Centrifuge



DNA solution

Autovortex Mixer


Breakup the RBC by lysis and SDS


DNA transferred

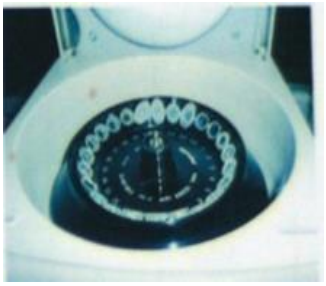
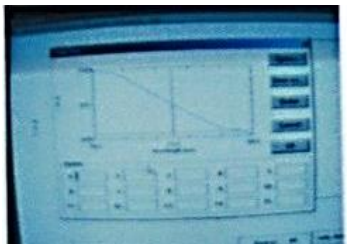

Microcentrifuge

Plate 3. Extraction of DNA


DNA pellet


Meaurement of DNA result


Resulted DNA

Plate 3 . Continued

RESULTS

The analysed individuals of the species, *Bos taurus* and *B. frontalis* were found to have a chromosome number of 2n = 60 and 2n = 58 respectively, within this sample were associated with two different karyotypes. *Bos taurus* type breed was found to have 58 acrocentric autosomes and two sexual chromosomes of a large submetacentric X chromosome and a small submetacentric Y chromosome. In domestic gayal or mithun *B. frontalis* were found to be two submetacentric autosomes (different in size) and 54 acrocentric autosomes in both sexes. The X chromosomes were the large submetacentric and the Y chromosome had the small submetacentric.

The chromosome (short arm and long arm) length, total length, centromeric index, arm ratio and chromosome morphology were measured from metaphasal plates and are presented in Table (1). The karyotype obtained after staining the chromosomes for G-bands is shown on Plate (4) to (7).

In homologous, dark bands and light interbands were noted at comparable position on both short and long arms. The chromosome morphology (centromeric position), observed in the cases studied consisted of three types; metacentric (M), submetacentric (SM), and acrocentric (A). Centromeres of the autosomes were unstained but those of the sex chromosome were darkly stained.

Extraction of DNA from blood samples of the studied species

Out of 14 blood samples (7 males and 7 females), 14 showed good DNA purity and concentration.

The genomic DNA extracted from the peripheral blood samples (5mL volume) were checked for purity and concentration.

The purity values between 1.7 to 2.0 (the ratio of absorbance of at OD_{260}/OD_{280}) was considered as the most purified DNA (Table 4,5 and Plate 3). There is no significant difference species *Bos taurus* and *Bos frontalis* at 1.826 and 1.799 of purity of OD_{260}/OD_{280}.

The concentration of extracted DNA at OD_{260} (from 5mL blood) samples was calculated as absorbance (at OD_{260}) 50 to obtain μg DNA/mL. (The standard is 50 μg/mL at OD_{260} = 1) (Table 4,5 and Plate 3).

The absorbance OD_{260}, OD_{280}, purity OD_{260}/OD_{280} of *B. taurus* has variate results while *B. frontalis* has low variate results (Table 4 and 5).

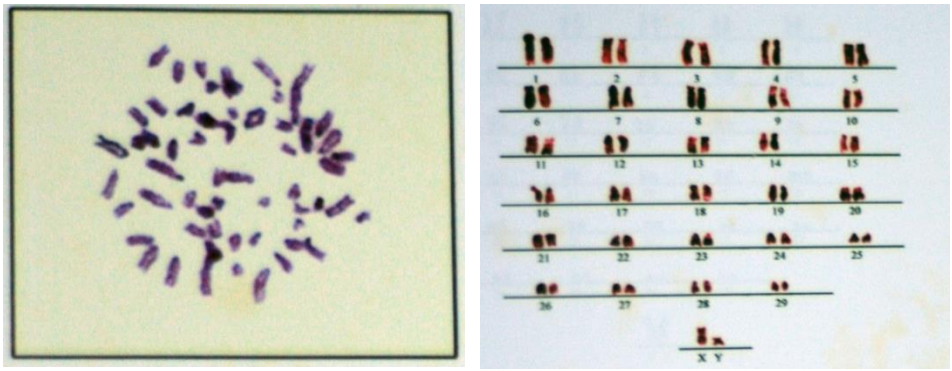


Plate 4. G-banding karyotype of male *Bos taurus* (2n = 60, XY)

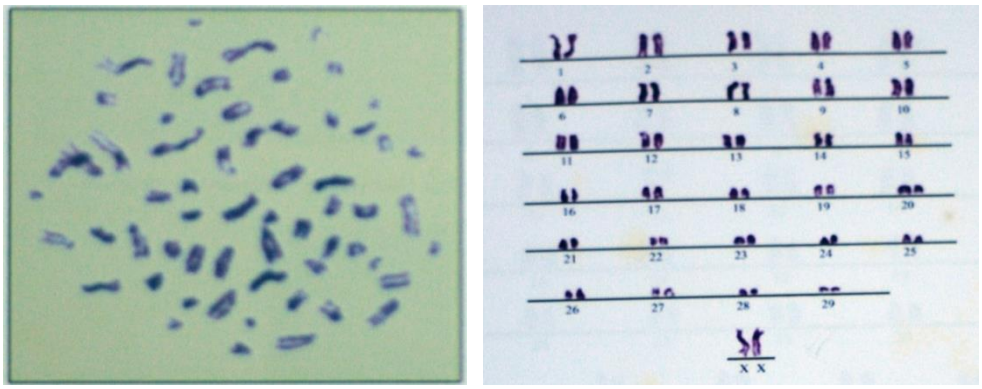


Plate 5. G-banding karyotype of female *Bos taurus* (2n = 60, XX)

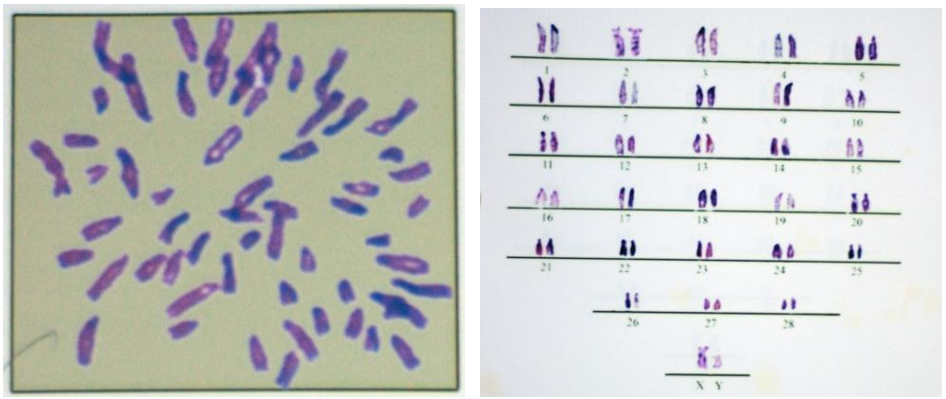


Plate 6. G-banding karyotype of male *Bos frontalis* (2n = 58, XY)

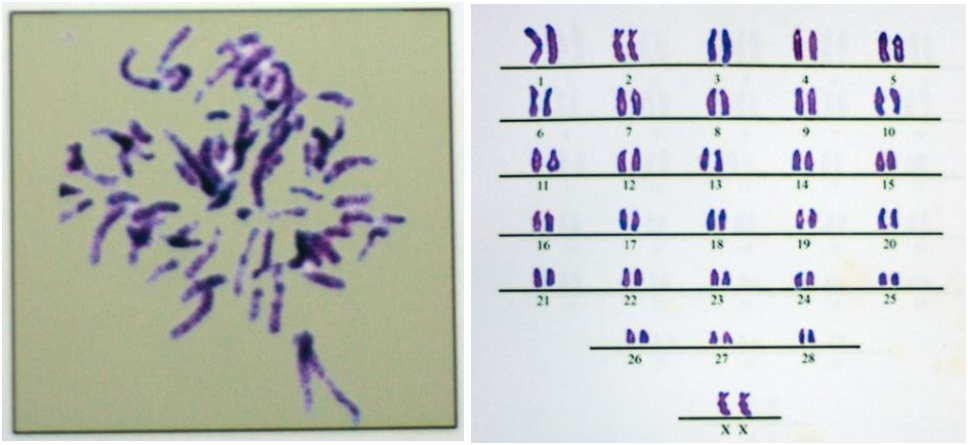


Plate 7. G-banding karyotype of female *Bos frontalis* (2n = 58, XX)

Table 1. Measurement of metaphase chromosomes of *Bos taurus* and *B. frontalis*

Chromo-some pair No.	*Bos taurus* (Male)		*Bos taurus* (Female)		*Bos frontalis* (Male)		*Bos frontalis* (Female)		Chromo-some type *B. taurus*	Chromo-some type *B. frontalis*
	2n=60		2n=60		2n=58		2n=58			
	AR	CI	AR	CI	AR	CI	AR	CI		
1	4.50	0.181	6.00	0.142	4.50	0.180	3.90	0.796	A	A
2	4.20	0.192	5.40	0.156	3.00 2.66	0.250 0.375	2.26 2.75	0.306 0.267	A	SM
3	4.60	0.180	4.80	0.172	4.30	0.233	5.00	0.166	A	A
4	4.40	0.183	4.60	0.178	4.00	0.200	5.71	0.148	A	A
5	5.12	0.163	6.00	0.142	3.90	0.204	5.57	0.152	A	A
6	5.00	0.166	4.40	0.227	4.40	0.183	7.00	0.125	A	A
7	5.42	0.155	4.00	0.200	5.00	0.166	6.00	0.140	A	A
8	6.50	0.133	5.25	0.160	6.70	0.130	5.60	0.179	A	A
9	6.00	0.143	5.00	0.167	6.50	0.133	5.40	0.185	A	A
10	7.00	0.125	6.70	0.130	5.80	0.146	5.20	0.192	A	A
11	7.00	0.125	6.33	0.136	7.00	0.125	5.20	0.192	A	A
12	7.00	0.125	6.33	0.136	7.00	0.125	5.00	0.167	A	A
13	6.60	0.132	5.70	0.150	6.80	0.128	6.50	0.133	A	A
14	6.00	0.142	5.70	0.150	6.40	0.135	6.50	0.133	A	A
15	5.60	0.152	3.75	0.210	6.00	0.140	6.25	0.138	A	A
16	6.80	0.129	3.75	0.210	5.80	0.147	6.00	0.142	A	A
17	5.00	0.166	5.30	0.157	5.60	0.179	5.75	0.148	A	A
18	6.50	0.133	5.30	0.157	5.40	0.185	5.50	0.154	A	A
19	4.80	0.172	5.00	0.167	5.20	0.192	5.50	0.154	A	A
20	4.60	0.178	5.00	0.167	5.00	0.166	5.30	0.160	A	A
21	5.30	0.160	4.66	0.176	5.00	0.166	5.30	0.160	A	A
22	5.00	0.167	4.33	0.188	6.25	0.138	5.00	0.167	A	A
23	6.70	0.130	6.50	0.133	5.75	0.148	6.70	0.130	A	A

24	6.33	0.136	6.00	0.142	5.50	0.154	6.33	0.136	A	A
25	6.00	0.143	6.00	0.142	5.30	0.160	6.00	0.143	A	A
26	5.70	0.150	5.50	0.154	6.70	0.130	6.00	0.143	A	A
27	5.00	0.167	5.00	0.167	6.30	0.136	5.70	0.150	A	A
28	6.50	0.133	4.50	0.181	6.00	0.143	5.70	0.157	A	A
29	5.50	0.154	4.00	0.200	-	-	-	-	A	A
X	2.50	0.288	1.90	0.343	2.33	0.300	3.00	0.750	SM	SM
X	-	-	1.90	0.343	-	-	3.00	0.750	SM	SM
Y	2.00	0.333	-	-	3.00	0.750	-	-	SM	SM

AR = Arm Ratio, CI = Centrometric Index, A = Acrocentric, SM = Submetacentric

Table 2. Mean total length of chromosomes in the studied two bovine species

Chromosome Pair No.	Mean length ± SE (μm)				
	Bos taurus (Male) n=10	*Bos taurus* (Female) n=10	*Bos frontalis* (Male) n=2	*Bos frontalis* (Female) n=2	Chromosome morphology
1	5.1 ± 0.66	4.96 ± 0.36	5.25 ± 0.25	4.7 ± 0.2	A
28	-	-	2.05 ± 0.05	1.15 ± 0.15	A
29	1.38 ± 0.31	1.2 ± 0.09	-	-	A
X	4.54 ± 0.5	3.78 ± 0.31	4.5 ± 0.5	3.9 ± 0.1	SM (Large)
X	-	3.66 ± 0.38	-	3.9 ± 0.1	SM (Large)
Y	2.88 ± 0.1	-	3.5 ± 0.5	-	SM (Small)

A = Acrocentric, SM = Submetacentric

Table 3. F Statistic Test results of comparisons between mean total length of some chromosomes in the two studied species

Chromo-some No.	Male			Female		
	F (5,2), $\alpha = 0.05$	P-value	Significant difference	F (5,2), $\alpha = 0.05$	P-value	Significant difference
1	4.58	P > 0.05	Non-significant difference	2	P>0.05	Non-significant difference
X	1.667	P > 0.05	Non-significant difference	6	P>0.05	Non-significant difference
Y	41.67	P < 0.05	Significant difference	-	-	-

Table 4. The purity and concentration of DNA extracted from the male studied species (N = 7)

Sr. No	Species	Absorbance OD_{260}	Absorbance OD_{280}	Purity (OD_{260}/OD_{280})	Concentration (μg/mL) (at OD_{260})
Male 1	*Bos taurus*	0.0496	0.0284	1.746	2.48
2		0.1250	0.0679	1.840	6.25
3		0.1508	0.0840	1.795	7.54
4		0.1925	0.1089	1.768	9.63
5		0.1427	0.0720	1.982	7.14
Male 6	*Bos frantalis*	0.1826	0.1034	1.765	7.13
7		0.1769	0.0965	1.833	8.85

(OD1 = 50μ g/mL DNA)

Table 5. The purity and concentration of DNA extracted from the female studied species (N = 7)

Sr. No	Species	Absorbance OD_{260}	Absorbance OD_{280}	Purity (OD_{260}/OD_{280})	Concentration (µg/mL) (at OD_{260})
Female 1	*Bos taurus*	0.1046	0.0612	1.709	5.23
2		0.0578	0.0315	1.841	2.89
3		0.2677	0.1512	1.771	13.39
4		0.0761	0.0407	1.870	3.81
5		0.2564	0.1403	1.828	12.82
Female 6	*Bos frantalis*	0.1035	0.0523	1.979	5.18
7		0.1414	0.0825	1.714	7.07

(OD1 = 50µ g/mL DNA)

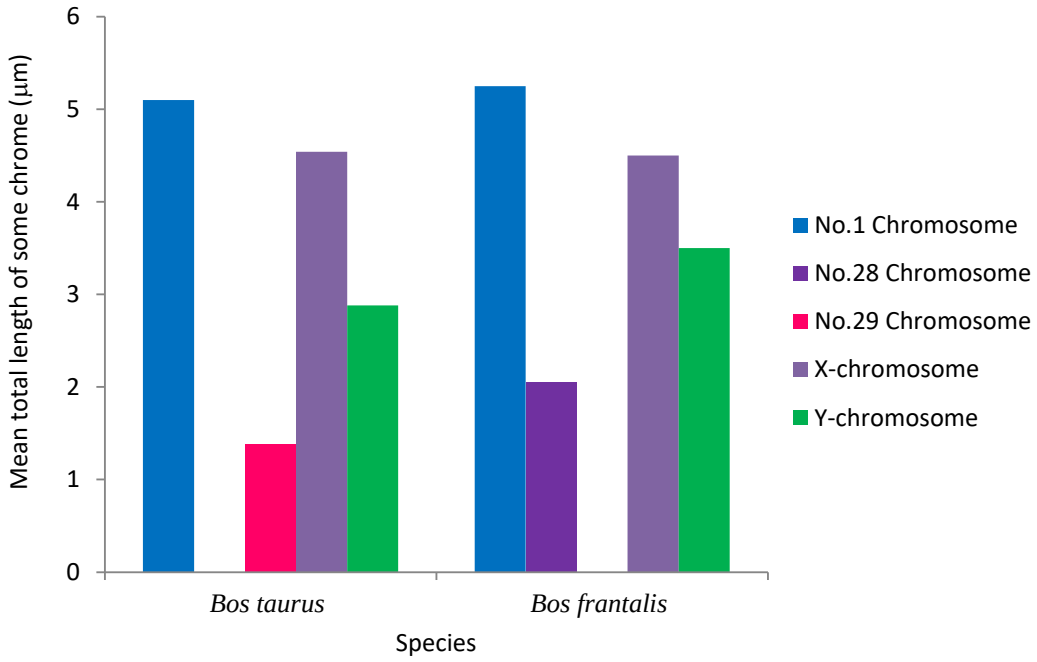


Fig 2. Mean total length of chromosomes in the male of the two bovine species

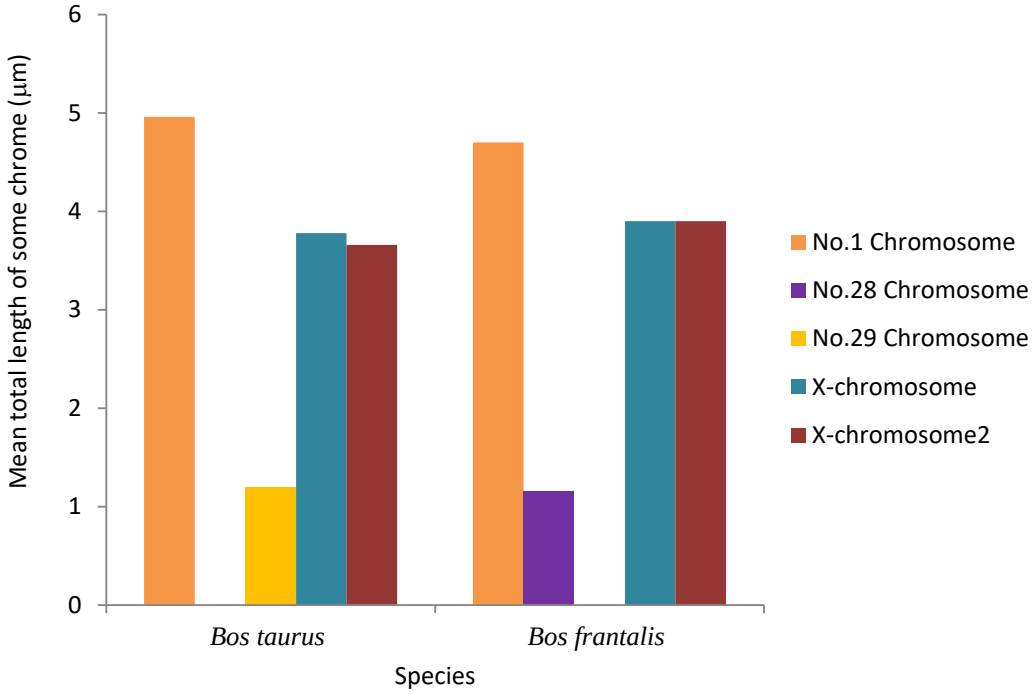


Fig 3. Mean total length of chromosomes in the female of the two bovine species

DISCUSSION

A total of 14 cases (seven males and seven females) were observed by G (Giemsa) banding method. In all these bovine species (*Bos taurus* and *B. frontalis*) of both sexes, metaphasal plates were seen clearly. Therefore, more accurate homologue chromosome pairing may be achieved by matching G-band similarly in the segments.

Iannuzzi, 1996 stated that the number of visible G-bands depends on the degree of chromosome condensation. Thus, the original reference ideograms that were made from standard G-banded metaphase chromosomes provided a schematic representation of domestic cattle chromosome types showing G-bands.

The chromosome number of domestic cattle (*Bos taurus*) were mostly 2n = 60, while domestic gayal or mithun (*Bos frontalis*) had 2n = 58 in this study.

Autosomal pairs in both sexes of *B. taurus* were acrocentric chromosome. In domestic gayal as mithun (*B. frontalis*) were found to be two submetacentric (different in size) and 54 acrocentric autosomes, most of which could be arranged in pairs in descending order of size.

Regarding the sex chromosomes, X chromosome of all females had submetacentric in the two bovine species. The X chromosomes were large submetacentric and Y had a small submetacentric chromosome in males of two bovine species.

In the present study, both sexes of the two bovine species, *Bos taurus* had 29 pairs of acrocentric autosomes. *B. frontalis* had one pair of submetacentric (No.2) and 27 pairs of acrocentric autosomes. The length of chromosome No.1 in both sexes of two bovine species was found to be non-significant difference ($p > 0.05$).

The X-chromosome in both sexes of the two bovine species were all submetacentric. The length of X chromosome in males and females of two bovine species was not significantly different ($p > 0.05$). The Y chromosome had a small submetacentric in males of two bovine

species. The Y chromosomes were found to be significantly different ($p < 0.05$), although the Y chromosome morphology was similar to the two different species.

The use of banding technique permits certain identification of each chromosome as well as mutations that alter neither the shape nor number of the chromosome. The purpose of the G-band examination in the present study was to find out the numerical and structural characteristics of the chromosome.

Gioffre *et al.,* 2004 mentioned that DNA bands bear a definite relationship to the linear array of the genes in the chromosomes. Several protocols have been used to prepare pure DNA samples for PCR.

The value was different in Y chromosome at two bovine species. There is a significant difference at 41.67 ($p < 0.05$) in total length of Y chromosome.

The method used for the DNA extraction and purification in this bovid study was the manual method used for human samples because humans and bovine species studied are in the same class Mammalia.

CONCLUSION

According to the result of the present study, DNA purity values showing the DNA extracts would be suitable for Polymerase Chain Reaction (PCR) analysis of the bovine peripheral blood DNA samples.

By studying the G-banded metaphase chromosome of the domesticated bovine species in Myanmar, precisely pure line breeds with economically beneficial traits could be selected and developed, and individuals with genetic abnormalities could be eliminated from existing livestock's in cattle farms.

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