Comparative genome integrity of riverine buffaloes (Bubalus bubalis) under tropical climatic conditions


Abstract The purpose of the present study was to evaluate the genome integrity of indigenous buffalo breeds - Murrah and Nilli-Ravi (same age, sex and environmental conditions) in the northern states Punjab (Patiala) and Haryana (Hisar & Karnal) of India. The climographs based on the monthly mean temperature (30 years) and monthly mean relative humidity of Hisar, Karnal and Patiala regions were prepared for assessment of climatic conditions of particular regions in which animals were reared throughout the year. Seventy five buffaloes of the two breeds were used in this study. All animals were kept in organized herd at three different farm locations. Comparative cytogenetic analysis was made between the two breeds of buffalo. The cytogenetic studies of peripheral lymphocytes included chromosomal aberrations (CAs) and sister chromatid exchange (SCE). The mean number of gaps in Murrah buffalo of Karnal and Hisar regions were 12.48±4.12 and 10.86±3.65 respectively. Similarly, the mean number of gaps in Nilli-Ravi breed of buffalo from Patiala was 7.0±2.27. The mean frequency of chromosomal breaks in Murrah buffalo from Hisar and Karnal were 5.96±1.92 and 5.44±2.72 and 3.1±1.97 in Nilli-Ravi respectively, the difference was statistically significant. The frequency (mean±SD) of sister chromatid exchanges/per cell in Murrah buffaloes from Hisar and Karnal were 6.64±2.21 and 6.26±1.92 and for Nilli-Ravi was 3.52±1.87. The results of cytogenetic analysis between the two buffalo breeds reared under same climatic conditions at different farm locations (Haryana and Punjab) reveal significant (P<0.01) differences in their genome integrity as detected by CAs and SCEs.

Keywords: Peripheral lymphocytes, buffalo, genome integrity

Introduction

Buffalo forms the principal dairy animal in the developing countries of Asia and mainstay of Indian dairy industry contributing over 54% of the total milk production. They also contribute significantly to the meat industry in Asia in general and India in particular. Buffalo has become an important animal in the agricultural economy of most of the Asian countries because of its resistance to disease, ability to convert poor quality roughage to meat and milk and its powerful draft capacity. In India, the preference for buffalo as milch animals has increased over the past few years as they are considered as better converters of fibrous feed into milk and better adapted to local climatic conditions.

Cytogenetic techniques have been used to evaluate the effect of environmental factors on the genome integrity. The basic technique used in chromosomal abnormality assessments is sister chromatid exchange (SCE) in addition to the techniques related to the detection of gaps and breaks (Iannuzzi et al. 2004). Sister chromatid exchange evaluation technique has been used to detect the genome stability in humans (Chaganti 1974) and farm animals for example cattle (Ciotta et al. 2005), goat (Di Berardino et al. 1979), sheep (Di Meo et al. 2000), buffalo (Ahmed, 1998), pig (Peretti et al. 2005) and horse (Wojcik et al. 2011). Cytogenetics in domestic animals was started in the early sixties and various abnormalities have also been reported in Indian cattle (Balakrishnan and Yadav 1984; Balakrishnan et al. 1985; Yadav 2000; Patel et al. 2005; Chauhan et al. 2009) and buffaloes (Yadav et al. 1987; Yadav et al. 1990) associated with reduced fertility or reproductive failure.
However, systematic cytogenetic investigation of environmental influence on buffalo genome integrity is still lacking and thus demands our attention. Therefore, the present study was carried out in order to assess the genome integrity of two buffalo breeds- Murrah and Nilli-Ravi thriving under the same climatic conditions at different farm locations of Haryana (Hisar and Karnal) and Punjab (Patiala). The aim of this study was to evaluate the genome integrity of the above mentioned breeds in their respective breeding tracts and also to verify if there are any differences in genome stability between the two breeds. In order to evaluate the genome integrity, chromosomal aberrations (CAs) and sister chromatid exchange (SCE) tests that are expressions for DNA damages were applied.

Materials and Methods

Selection of animals and blood sample collection

A total of seventy five animals from two different buffalo breeds (Murrah and Nilli-Ravi) were selected for the study from three organized herds maintained at National Dairy Research Institute, Karnal (Haryana), Central Institute on Buffalo Research, Hisar, (Haryana) and a regional buffalo research station at Nabha,(Punjab). Blood samples were collected in heparinised vacutainer tubes by jugular vein puncture and brought to laboratory below 8°C and stored at 4°C till used for setting up cultures. The study was carried out as per guidelines issued by the Institute Animal Ethics Committee (IAEC).

Chemicals and reagents

Most of the chemicals and media used were of high purity and culture grade (Sigma-Aldrich, USA) and other standard brand names. Heparanized vacutainers and needles were purchased from Becton Dickinson (USA). Foetal Bovine Serum (FBS) was taken from Hyclone, (USA).

Whole blood-lymphocyte culture for chromosome preparations

The blood samples collected were used for lymphocyte culture and preparation of chromosomes by routine method (Balakrishnan and Yadav 1985). Chemicals used and steps followed are briefly described. Bulk culture medium was prepared, which included RPMI 1640 supplemented with 10 % fetal bovine serum, penicillin (20 IU/ml), streptomycin (20 μg/ml) and phytohemagglutinin (10 μg/ml). It was distributed into 30 ml capacity culture tubes each with 6 ml volume and stored at -20°C till used. An aliquot of 0.5 ml whole blood was added per culture tube at room temperature in a clean air laminar flow hood. Usually four replicate cultures were established for each animal. All the culture tubes were incubated for 72 hours at 37.5±0.5°C. Colchicine (10μg/ml) was added 45 minutes before harvesting of the cultures. Harvesting of cells was done in 15 ml centrifuge tubes and centrifugation was carried out at 1800 rpm for 15 minutes. Supernatant was discarded and the packed cells were treated with hypotonic salt solution (0.075M KCl) for 8 minutes in a water bath at 37°C. Subsequently the action was stopped by addition of 1 ml chilled fixative [methanol: acetic acid (3:1)] to each tube. The cells were washed three times with freshly prepared chilled fixative. The cell suspension was incubated overnight at -20°C.

Slide preparation

The chromosomes preparations were obtained by routine method used with some slight modifications. After overnight incubation at low temperature, the cell suspension was washed with fresh fixative two times. Chromosomes preparations were made by putting from Pasteur pipette 3-4 drops on clean glass slides from 2-3 feet height.

Staining of slides with Giemsa solution

The air dried slides were stained with Giemsa solution. The slides were placed in freshly prepared 2 % Giemsa solution at pH 6.8 for 25 minutes, and the excess stain was removed by washing three times with distilled water.

Lymphocyte cultures for evaluation of SCEs

For revelation and evaluation of SCEs, the culture medium and conditions used were same as described for chromosome aberration, however, with minor modifications (Perry and Wolff, 1974). In the culture medium during incubation after 24 hours of setting up of culture 10 μg/ml of 5-bromo-2'-deoxyuridine (BrdU) was added and incubated further for next 48 hours. Subsequently, the FPG (Fluorescence plus Giemsa) method of chromosome staining was used. The slides were aged for 7 days then stained in 50 μg/ml Hoechst 33258 dye for 30 min. The slides were rinsed in distilled water, layered with Mcllvaine buffer (di-sodium hydrogen phosphate and citric acid, pH 7.5).Then slides were placed below a ‘black blue tube light’ at distance of about 2 inches for 55 minutes, this step was done keeping the assembly in an incubator set at 50°C. Slides were rinsed in Mcllvaine buffer, then immersed in Giemsa solutions (4 ml stock diluted with 18ml Mcllvaine buffer and 78ml distilled water) for 5 minutes and rinsed with tap and distilled water.

Examination of Giemsa stained and FPG exposed metaphase stage chromosomes

The stained slides were screened under microscope (Leica DMRB) for evaluation of chromosome numbers, gaps, breaks, SCE and gross anomalies. For each animal, 50 good quality
metaphases were examined, photographed and the data were recorded for analysis.

Meteorological parameters

The monthly average air temperature and relative humidity of Karnal, Hisar (Haryana) and Patiala (Punjab) regions were recorded as climograph, based on the last thirty years of climate data (Indian Meteorological Department, Pune). The temperature humidity index (THI) used in this study was calculated using Thom (1959) equation:

\[0.72 \left( T_{db}^\circ C + T_{wb}^\circ C \right) +40.6\]

Statistical Analysis

The data were analysed using one-way ANOVA (GraphPad Prism version 5.00, USA).

Results and Discussion

The mean frequency of chromosomal aberrations including gaps and breaks in each breed of buffalo are shown in Figure 1. The frequency of gaps in buffalo from Hisar, Karnal and Patiala regions was 12.48±4.12, 10.86±3.65 and 7.1±2.27 respectively (Figure 1). The range of gaps was 3-14, 3-16, 1-8 per 50 cells in the Murrah buffalo from Hisar, Karnal and Patiala respectively. The mean frequency of breaks in buffalo from Hisar, Karnal and Patiala regions was 5.96±1.92, 5.44±2.72 and 3.1±1.97 respectively (Figure 1). Results indicated that the range of breaks in Murrah buffalo from Hisar, Karnal and Patiala was 0-6, 0-7 and 1-6 per 50 cells respectively.

In the present study, the frequency of sister frequency chromatid (SCE) in Murrah and Nilli-Ravi from Hisar, Karnal and Patiala regions was also carried out. The mean frequency of sister chromatid exchange (SCE) in each breed of buffalo is shown in Figure 2. The frequency of SCE in buffalo from Hisar, Karnal and Patiala regions was 6.64±2.21, 6.26± 1.92 and 3.52±1.87 respectively (Figure 2). Similarly the range of SCEs was 1-9, 2-9 and 1-7 per cell in buffalo breeds from Hisar, Karnal and Patiala regions respectively. The difference between total chromosomal aberrations was significant (P ≤0.01) in Murrah buffalo from Hisar, Karnal and Patiala regions.

Statistical analysis revealed significant differences between mean frequency of SCEs in Murrah and Nilli-Ravi breeds of buffalo from all the three regions. The metaphase plates showing the SCE and the CAs in the genome of Murrah buffalo from Hisar, Karnal and Patiala region are shown in figures 3, 4 and 5 respectively.

In order to compare the climatic conditions of the three regions (Hisar, Karnal and Patiala), information on the monthly average temperature and precipitation of these locations was gathered from Indian meteorological department (IMD, Pune), and climographs for these regions were prepared based on this information (Fig. 6, 7 and 8). The temperature humidity index (THI) for the three climatic regions has been represented in Table 1.

The results obtained here can also be extended to previous studies carried out in Egyptian buffaloes by Ahmad et al. (1998) who studied the effect of environmental pollutants on thirty four Egyptian buffaloes. They reported a significantly higher mean frequency of SCEs in the buffalo samples exposed to environmental toxicants (11.8±1.4) compared to the control

<table>
<thead>
<tr>
<th>Climatic zones</th>
<th>Zone type</th>
<th>Buffalo breeds</th>
<th>T_{db}^\circ C</th>
<th>T_{wb}^\circ C</th>
<th>THI</th>
<th>RH(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hisar</td>
<td>Semi-arid</td>
<td>Murrah</td>
<td>25.85</td>
<td>18.8</td>
<td>72.74</td>
<td>52.0</td>
</tr>
<tr>
<td>Karnal</td>
<td></td>
<td>Murrah</td>
<td>25.0</td>
<td>18.55</td>
<td>71.96</td>
<td>56.5</td>
</tr>
<tr>
<td>Patiala</td>
<td></td>
<td>Nilli-Ravi</td>
<td>25.1</td>
<td>18.45</td>
<td>71.95</td>
<td>54.5</td>
</tr>
</tbody>
</table>

The group of buffalo investigated in this study was very homogeneous (same age, sex, same environmental conditions). In the present study, cytogenetic analysis revealed significant (P≤0.01) instability in the genome of Murrah and Nilli-Ravi animals in their respective breeding tracts as detected by CAs and SCE. The results revealed significant (P≤0.01) differences in the frequency of CAs and SCEs between the two breeds. The representative riverine buffalo genome investigated by the SCE test was reported by Iannuzi et al. 1988, who studied 34 buffalo samples reared in southern Italy. They found a mean frequency value of 8.8±3.4 SCE/cell which is considerably higher than the mean values of 6.64±2.21 (Murrah) and 3.52±1.87 (Nilli-Ravi) observed in the present study.

The present values are close to the results reported in Bhadawari buffaloes (5.56 per cell) and Murrah buffaloes (3.66 per cell) (Vijh et al. 1991; Vijh et al. 1995). However, the mean frequency of SCE/cell obtained in the present study for both the buffalo breeds is lower than Surti (14.05 ± 0.12) (Murali et al. 1998) and Toda (7.8±0.23) breed of buffalo (Murali et al. 2009).

The present values are close to the results reported in Egyptian buffaloes by Ahmad et al. (1998) who studied the effect of environmental pollutants on thirty four Egyptian buffaloes. They reported a significantly higher mean frequency of SCEs in the buffalo samples exposed to environmental toxicants (11.8±1.4) compared to the control
value of 8.3±1.2. Therefore, the results of SCE for Murrah and Nilli-Ravi buffalo breeds obtained in the present study are more closer to the control values obtained by Ahmad et al. (1998). In the present study, a low frequency of SCEs and CAs was observed for Nilli-Ravi and Murrah compared to
previous studies in Bhadawari, Surti and Toda buffaloes breeds (Murali et al. 1998; Murali et al. 2009; Vijh et al. 1995), thereby, suggesting that their genome has been strongly selected and more stable in comparison with other breeds of the same species.

In another study, a baseline frequency of 8.3 ± 1.1 and 7.76 ± 0.8 for SCE in Beheri and Saidi breed of Egyptian water buffaloes has been described (Ahmad et al. 2001). The results obtained here are well below this baseline suggesting the more stable genome integrity in Murrah and Nilli-Ravi. Our results are also confirmed by the study of Pires et al. 1998 who studied the CAs in one hundred and eighty two Murrah, Mediterranean and Jaffrabadi buffalo breeds reared in the state of Sao Paulo, Brazil. They observed a mean value of 3.01, 2.49 and 3.89 percent CAs in Murrah, Mediterranean and Jaffrabadi breeds of buffalo respectively. The present values are also close to these values observed by Pires et al. 1998 for the respective buffalo breeds.

The current study stands in agreement with Ianuzzi et al. (1991) who studied the sister chromatid exchange in chromosomes of three different cattle breeds viz. Podolian, Friesian and Romagna reared under similar conditions in Italy. They reported only small differences in the mean values of SCEs/cell between the Podolian and the Friesian breeds. Similar results are obtained in this study where significant difference in the mean values of CA and SCE was observed between the two breeds of buffalo. Wojcik et al. (2011) described the significant breed effect on the frequency of SCEs in the groups of six horse breeds. Their results depicted the significant variation in the mean values of SCE between all the six breeds. Ciotola et al. (2005) also reported the significant differences in the SCE frequency between the Agerolse, Podolian, Romagna and Holstein Friesian cattle breeds. Peretti et al. (2006) also concluded that the breed had a significant effect by comparing the SCE frequency of Casertana pig breed analysed in his study with the Landrace breed described by Rubes et al. 1987.

However, the present results stand contrary to the studies carried out in sheep (Di Meo et al. 2000) and humans (Chaganti 1974). Di Meo et al. (2000) reported no significant differences in SCE level between Comisana and Laticauda breeds of sheep, thereby nullifying any effect of the breed/race on SCE frequency. No significant variations concerning the SCE level have been observed when different human races were compared (Butler et al., 1981; Wulf et al. 1986).

**Conclusions**

Therefore, in this study the results obtained indicate the better adaptive capacity of Murrah and Nilli-Ravi buffalo breeds to withstand tropical climatic conditions in their native home tract and perform reasonably well even with low inputs of fodder availability. However, the SCE and CA level differed significantly between Murrah and Nilli-Ravi, with Murrah breed showing the significantly higher SCE/cell and CAs compared to Nilli-Ravi. Therefore, it can be concluded that this better capacity in Nilli-Ravi as revealed by the lower mean frequency of CAs and SCEs has been developed through long-term natural selection and evolution over the centuries. The variations in the number of CAs and SCEs between the two buffalo breeds could be due the different intensity of selection, the prevailing demographic factors, management strategies and agricultural traditions. The observations seem to be relevant
particularly in the present scenario of changing climate. The naturally evolved breeds of Murrah and Nilli-Ravi can be used as invaluable genetic resources that exhibit a better adaptation to extreme climatic conditions. In view of scarcity of literature on the genome integrity of Murrah and Nilli-Ravi breeds of riverine buffalo, this information can serve as a baseline for future investigations. The observations made in the present study and the existing literature on other breeds of buffalo suggests that the CAs and SCEs in the chromosomes of riverine buffaloes have a wide range contributing to their superior adaptability and productivity traits that can be exploited in this scenario of climate change.

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