Annals of Carcinogenesis

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Austin Publishing Group

Rapid Communication

Cytogenetic and Genomic Investigations in River Buffaloes Raised in Farms Located in Urban and Rural Areas of Campania Region (Southern-Italy)

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Received: October 29, 2019; **Accepted:** November 28, 2019; **Published:** December 05, 2019

Abstract

This study aimed to check possible differences between long- and short-term DNA damages in lymphocytes of river buffalo cows raised in urban and rural areas by both cytogenetic and genomic tests. Two groups of buffaloes, homogeneous for age, sex and feeding, were studied: group A (Naples district) was raised in an urban area, while group B (Salerno district) was raised in a rural area. Three long-term DNA damage tests (CA, SCE and CBMN) and one short-term DNA damage test (RLTL) have been performed on both groups. Interestingly, no statistical differences were found between the two groups for each test, supporting the possible restarting of normal environmental conditions in the urban area, considered potentially polluted in the past.

Keywords: River Buffalo; Cytogenetic Tests; Genomic Test; Environment; Pollution

Abbreviations

CA: Chromatid and Chromosome Breaks; SCE: Sister Chromatid Exchange; CBMN: Cytokinesis-Block Micronucleus; RLTL: Relative Leukocyte Telomere Length; CCL: Concentration of Contamination Level; BCI: Binucleated Cell Index; MN: Micronuclei, BN: Binucleated; MMQPCR: Monochrome Multiplex Quantitative PCR; STL: Sperm Telomere Length; SCG: Single Copy Gene; NTC: No Template Control

Introduction

Several pollutant types, partially volatile and derived from human activities, even if present at low concentration in the environment, can interfere with physiological systems and therefore the capacity of ruminants and other animals to reproduce, rear offspring or fight disease. Indeed, the exposition to exogenous agents, alone or in combination, can lead to a variety of modifications on DNA composition, resulting in genome and chromosome alterations. Chromosomes are still considered one of the best biological markers to monitor damages associated with natural or *in vitro* exposure to environmental mutagens. Recently, in addition to routine cytogenetic tests such as CA, SCE and CBMN [1-3], also a genomic test has been used. The analysis of the RLTL (expressed as telomere length relative to a single copy reference gene) has been performed to check DNA-damages in human populations exposed to pollutants in both leucocytes [4] and sperms [5]. For this reason, the monitoring of livestock population by cyto-genomic tests might represent a good tool to indirectly control of the food chain, to preserve health problems, and to avoid management and income issues at the farm level.

Several studies have investigated the mortality rates in the polluted areas of the Campania region highlighting an increased level of mortality in the human population of Naples and Caserta districts, compared to the remaining ones (Avellino, Benevento, and Salerno) [6]. However, the CCL analyses, recently performed by the official Environmental Regional Agency of Campania region (ARPAC http://www.arpacampania.it), within different areas of Naples and Caserta districts, reported that only the 6.2% of the areas earlier retained polluted are now forbidden for agro-food production [7].

The study is also a comparison between long- (cytogenetic) and short- (genomic) term tests to be applied for environmental assays. In our knowledge, this is the first time that this type of study has been performed in domestic animals.

Materials and Methods

Two groups of Italian Mediterranean river buffaloes (20 animals per group), homogeneous for age (2-3 years old) and sex (females), randomly chosen from two farms have been selected for the analysis. The two farms used also similar diet and vitamin supplementation and were located in two different areas of the Campania region: the group A in a urban area of Naples district with high environmental pressure in the past, and the group B located in an rural area at low environmental pressure (Salerno district), considered as control (Figure 1). The study was also approved by the Ethical Commission of the National Research Council (CNR), ISPAAM of Naples (reg. n 653 of June 5, 2017).

We have performed three cytogenetic (CA, SCE and CBMN) and one genomic (RLTL) tests. The CA and SCE tests have required peripheral blood cultures, hypotonic treatment, fixations and acridine orange stain [8]. 100 and 35 cells (with entire chromosome set, 2n=50) for each animal, were studied for CA- and SCE-tests, respectively [9]. For CA-test, only no linear breaks were considered (Figure 2). The CBMN test has required different blood cultures, hypotonic treatment, fixations and Giemsa stain [10]. The nuclear division index and the percentage of BCI were examined for each sample. The

Citation: Iannuzzi A, Perucatti A, Genualdo V, Rossetti C, Iorio C, Caputi Jambrenghi A, et al. Cytogenetic and Genomic Investigations in River Buffaloes Raised in Farms Located in Urban and Rural Areas of Campania Region (Southern-Italy). Ann Carcinog. 2019; 4(1): 1020.

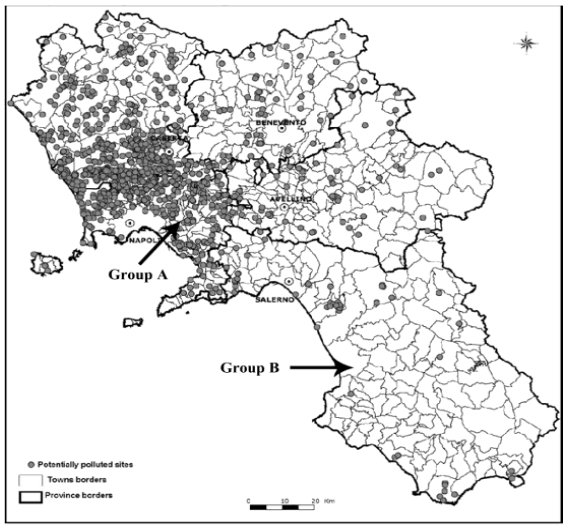

Figure 1: Geographic representation of Campania region (southern-Italy) with the indication of areas earlier retained potentially polluted by the official ARPAC agency (http://www.sito.regione.campania.it/burc/pdf05/burcsp09_09_05/pianoregionale_bonifica.pdf). Arrows indicate the locations of the two farms used for the study: one in a urban area (Group A) and the other one (Group B) in a rural area.

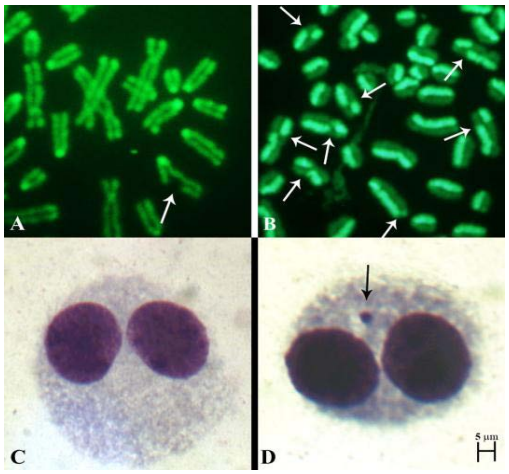

Figure 2: Details of CA (A), SCE (B), BC (C) and MN (D) in river buffalo lymphocytes.

analysis of MN was performed on 1000 BN cells, for each sample, with preserved cytoplasm (Figure 2). The RLTL test was performed by MMQPCR according to the method described by Cawthon et al. [11] (Figure 3). The RLTL was measured as T/S ratio (average ratio of telomere repeat copy number to a scg) for each sample and calculated using the ΔΔCt method: T/S ratio= $2^{-(\Delta Ct_{telomere}-\Delta Ct_{scg})} = 2^{-\Delta\Delta Ct}$ [12].

For each animal and animal group, it has been estimated the mean values and the standard deviation. The mean value of each test was compared by the t-student test, and Bonferroni correction was applied as a default restriction. Differences were considered significant for $P < 0.05$.

Results

Data obtained for the two groups of buffaloes are summarized in Table 1. No significant differences were found between the two examined groups of buffaloes comparing the results obtained by four different tests, of which three at long- (CA, SCE and CBMN) and one (RLTL) at short-term of DNA damage.

Table 1: Mean values of CA, SCE, CBMN (BCI and MN) and RLTL (T/S ratio) in the two groups of river buffalo cows reared in farms from urban (A) and rural (B) areas of Campania Region.

PARAMETERS	Group A	Group B	(p-Value)
CA	0.06±0.26	0.07±0.27	0.48
SCE	8.96±3.86	9.18±4.35	0.32
CBMN BCI	76.70±7.32	74.89±6.32	0.75
MN	1.52±1.87	1.76±2.07	0.73
T/S ratio	1.34±0.92	1.44±1.21	0.78

a, b Values within columns with different superscripts are significantly different; P< 0.05.

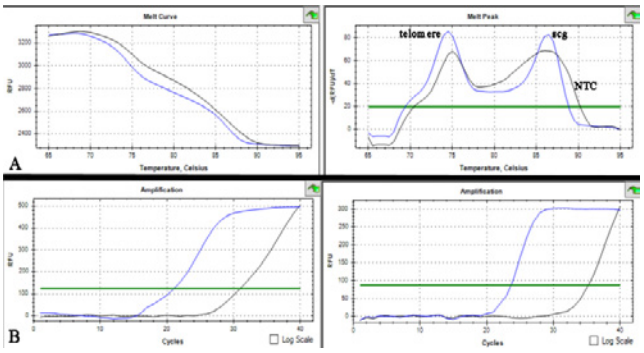

Figure 3: (A) Representative dissociation curve (blue) for telomere and SCG using CFX RT-PCR System. The 74°C read corresponds to the Ct value for the telomere amplification while the 87°C read corresponds to the Ct (cycle threshold) value for the scg amplification. (B) Representative amplification Ct curve for telomere (left) and scg (right). NTC is also reported (black).

Discussion

It is interesting to note that all four tests gave similar results between the two groups (Figure 4), suggesting that the RLTL test could be very useful for environmental analyses, being faster than other tests and no requiring cell cultures but only DNA-extraction and RT-PCR analyses. Our results agree also with recent studies performed in human males living in areas of the Campania region at high and low environmental pressure [13]. Indeed, these authors did not identify statistical differences when analysing RLTL, but they found statistical differences when examined for STL. The authors could not explain these data, probably due to a large variability of age of male samples (18-35 years), the low number of subjects and RLTL age-dependent attrition in comparison to STL, longer in older human sperm donors than in younger ones [14]. The two groups of buffaloes investigated in our study showed no sensitivity to the different environmental pressure. We could hypothesize that our data are likely to represent also those in other areas of Naples and Caserta districts, earlier reported with a high environmental pressure (Figure 1). Indeed, the most recent data published by the ARPAC on the CCL over the permitted value, with at least one contaminant and performed upon 145 hectares of soil in areas of Naples and Caserta provinces earlier found polluted, 101 hectares (67.4%) were found suitable for the agro-food production and only 9 hectares (6.2%)

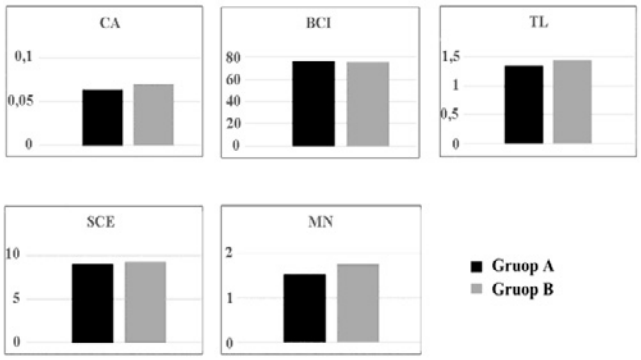

Figure 4: Graphic representation of the two river buffalo groups reared in farms from urban (A) and rural (B) areas of the Campania region.

resulted forbidden for both agri-food and pasture. The remaining 35.2 hectares have been classified suitable for agriculture use with some limits for some agro-food production in specific conditions [7]. Furthermore, the Napoli district area, considered in this experiment, has been classified as a low-environmental impact area in 2014.

Conclusion

Further cyto-genomic analyses, on both leucocytes and sperms, should be performed in larger samples (and areas) of both animals and humans living in different environmental conditions to fully confirm the environmental improvements that occurred in these two districts covering a large area hosting about 3 millions of people. RLTL-test could be largely applied on both humans and animals exposed to environmental pressures.

Conflict of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgments

The authors wish to thank Mr. Domenico Incarnato (CNR-ISPAAM of Naples), for excellent technical support. The study has been partially supported by the project, Genobu PON1_486.

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