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Science of the Total Environment xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Analysis of chromosome damage by sister chromatid exchange (SCE) and redox homeostasis characterization on sheep flocks from Sardinian pasturelands

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HIGHLIGHTS

- SCE test was used to test the genome damage of Sardinian sheep for the first time.
- Significant SCE means were found in three groups of polluted areas.
- Plasma levels of Asc, Toc and Ret were significantly lower in the exposed sheep.
- N-Tyr, PC, and LPO levels were lower in the control than in the exposed groups.

ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form 23 April 2015
Accepted 6 May 2015
Available online xxxx

Keywords:
Sister chromatid exchanges
Sardinian sheep
Environmental pollution
Chromosome fragility
Redox homeostasis
Bio-monitoring

ABSTRACT

Over the last decades, an increase of pollutants of diverse origin (industrial, military, mining, etc.) was recorded in several areas of Sardinia Island. We report the results of a multidisciplinary and complementary study based on cytogenetic and physiological analyses. The data obtained show the effects of the environmental impact on six sheep flocks (Sardinian breed) grazing on natural pasturelands next to possible polluted areas and compared to three herds grazing in different areas far from those potentially contaminated and used as control. Sister chromatid exchange (SCE) test was used as cytogenetic test to analyze chromosomal damages and it was performed on peripheral blood samples collected from 129 adult sheep (age > 4 years) randomly selected from polluted (92 animals) and control (37 animals) areas. Two types of cell cultures were performed: without (normal cultures) and with the addition of 5-BrdU. SCE-mean values estimated over 35 cells counted for each animal were 8.65 ± 3.40, 8.10 ± 3.50, 8.05 ± 3.08, 7.42 ± 3.34, 9.28 ± 3.56 and 8.38 ± 3.29 in the exposed areas, whereas the average values were 7.86 ± 3.31 in the control group. Significant increases ($P < 0.01$) of SCEs were found in three investigated areas of Southern Sardinia. Furthermore, sheep of the same flocks were characterized for blood redox homeostasis in order to define the potential targets of oxidative damage and to identify biomarkers of the extent of animal exposure to environmental contaminants. The plasma levels of Asc, Toc and Ret were found to be significantly lower ($P < 0.001$) in exposed sheep (I, II, IV and V) than in the control group. TAC as well as GPx and SOD activities were higher in control than in the exposed groups ($P < 0.001$). Finally, plasma levels of N-Tyr, PC, and LPO were significantly lower ($P < 0.001$) in the control group than in the exposed groups.

1. Introduction

Sardinia is the second largest island in the Mediterranean Sea, well known for its natural landscapes and for its endemic species. The island is considered as a major center of plant diversity. Despite that, various areas of this region are at environmental risk due to the contamination and/or pollution generated by civil and industrial activities.

Abbreviations: SCE, sister chromatid exchange; 5-BrdU, 5-bromodeoxyuridine; ROS, reactive oxygen species; Ret, Retinol; Toc, alpha-Tocopherol; Asc, Ascorbic Acid; N-Tyr, nitrotyrosine; PC, protein-bound carbonyl; LPO, lipid hydroperoxide; SOD, superoxide dismutase; GPx, glutathione peroxidase; TAC, total antioxidant capacity; ELISA, enzyme linked immunosorbent assay; HPLC, high performance liquid chromatography.

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http://dx.doi.org/10.1016/j.scitotenv.2015.05.021
0048-9697/

The most polluted areas are localized in the southwest of the Island (Boni et al., 1999; Sanna et al., 2003; Beccaloni et al., 2013) where high concentrations of volatile organic compounds (benzene, formaldehyde, xylene), polycyclic aromatic hydrocarbons and heavy metals have been found as a result of metallurgic industrial and mining activities (Peluso et al., 2013; Madeddu et al., 2013; Varrica et al., 2014; Cidu et al., 2014). In the same areas, residues of chemical emission due to military tests have been also found (Cristaldi et al., 2013).

A total of 18 areas assessed as industrial, mining, military and urban zones have been considered at environmental risk in this Island (Biggeri et al., 2006).

In this context, it is worth mentioning that noxious pollutants, such as sulfur oxides, nitrogen oxides, hydrocarbons, carbon monoxide, and transition metals, may induce DNA damage and genome mutations exerting carcinogenic effects (Goulart et al., 2005; Mateuca et al., 2005; Cavallo et al., 2008). These pollutants also promote highly reactive oxygen species (ROS) production (Matés et al., 2010), and depression of several ROS quenching systems (Ishida et al., 2009), with subsequent accumulation of toxic compounds in blood and tissues (Knerr et al., 2006). In physiological conditions, the antioxidant defense system, provided by enzymes and antioxidants, scavenges ROS thus limiting or preventing oxidative damage (Halliwell, 2012). The imbalance between ROS production and neutralizing capacity of antioxidant mechanisms may lead to oxidative stress (Cadenas and Davies, 2000; Halliwell and Gutteridge, 2000), which is associated with modifications of physiological and metabolic functions (Halliwell and Gutteridge, 2000).

In the last decades, the epidemiological data on the incidence of human pathologies analyzed in Sardinia have showed a general increasing trend, as well as rising mortality rates have been reported for the most prevalent types of cancer in both sexes (Budroni et al., 2013).

A recent investigation of the National Association of Italian Veterinarians (FNOVI) reported a correlation between the incidence of cancers in sheep farmers and the emergence of genetic malformations in newborn lambs located in potentially polluted areas (Mellis and Lorrai, 2013). In this respect, the farm animals represent good environmental sentinels (especially those naturally pastured) to facilitate the assessment of human exposure to environmental contaminants. For instance, they can be used as a monitoring system to reveal early environmental contamination, to monitor contamination of the food web, and to investigate the presence of contaminants in environmental media (van der Schalie et al., 1999).

Among farm animals, sheep are particularly suitable to be used as environmental sentinels. The reason for that lies in their feeding characteristics. In fact, differently from the other ruminants, sheep are raised on pasture and about 12% of their daily feed is represented by soil. Pollutants are accumulated mostly in soil and their permitted values are a far times higher than those admitted in plants (i.e. dioxin's values are 0.75 ng/kg and 10 ng/kg in plants and soil, respectively).

Cytogenetic tests represent direct and sensitive methods that have been used for detecting DNA damages in chromosomes for the bio-monitoring of different species (Penders et al., 2012; Wójcik and Smalec, 2013; Yang et al., 2014). In particular, SCE is a short-term test for the detection of reciprocal exchanges of DNA between two sister chromatids, involving DNA breakage and subsequent re-junction. The increased frequencies of SCE due to negative conditions, like pollutant exposition, lead to DNA single strand breaks as reported in several studies on livestock populations (Rubes et al., 1997; Di Meo et al., 2000, 2011; Iannuzzi et al., 2004; Perucatti et al., 2006; Genualdo et al., 2012; Wójcik and Smalec, 2013). The goal of this study is to evaluate the impact of environmental pollutants in some areas of Sardinia Island on the genome of sheep flocks living in this region by using the SCE test. Furthermore, as the analysis of blood redox homeostasis has become an important complementary tool for the evaluation of health and metabolic status of dairy cows (Bernabucci et al., 2005; Castillo et al., 2003, 2005, 2006), and feedlot calves (Castillo et al., 2012), we also characterized blood redox homeostasis of sheep in order to define the potential targets of oxidative damage in blood, and to describe physiological changes associated with exposure to pollutants.

2. Materials and methods

2.1. Farm selection

The different areas were selected according to the chemical emissions of different activities as reported in former investigations (Table 1).

The cytogenetic study was performed in Sardinia on 129 adult sheep (age > 4 years) of Sardinian breed, 92 grazing on natural pasturelands near possible polluted areas selected as follows: two herds (~12 sheep for each herd) located in the Northern area (industrial and military zones) and four flocks (~15 sheep for each flock) located in the Southern area (military, industrial and mine zones) of the island. For comparison, 37 sheep, reared in three different herds, were randomly selected in areas far from possible polluted zones and used as control (Fig. 1).

Furthermore, physiological investigations were performed on 80 exposed sheep (20 per each group) and 20 sheep used as a control group belonging to the same farms.

2.2. Cell cultures

Whole blood samples were collected from the jugular vein using sterile Vacutainer tubes containing sodium heparin as anticoagulant. About 1 ml of whole blood sample was added to the culture mix composed of 7 ml of RPMI medium, enriched with fetal calf serum (20%), L-glutamine (0.25%), antibiotic–antimycotic mixture (0.5%) and concanavalin A (20 μg/ml) as mitogen. Culture flasks were incubated at 37.8 °C for 72 h. Cell cultures from the investigated animals were

Table 1
Chemical emissions due to the various activities of selected areas of Sardinia Island.

Areas	Activity	Emissions	References
Control	Agriculture	n.a.	
Exposed I	Mining	Ag, Ba, Cd, Cr, Ni, Pb, Rb, Sb, U, V, Zn	Pirastu et al. (2011) Safronova et al. (2012) Madeddu et al. (2013) Varrica et al. (2014)
Exposed II	Military	Former nuclear submarine base	Aumento et al. (2005) Hernandez et al. (2011)
Exposed III	Industrial	Hg, Cd, Pb, Zn	Schintu and Degetto (1999) Sanna et al. (2003)
Exposed IV	Industrial	IPA	De Luca et al. (2004) Pirastu et al. (2011)
Exposed V	Military	Rb, Tl, W, Ti and Al, Cd, Pb	Cristaldi et al. (2013) Gatti et al. (2013)
Exposed VI	Military/industrial	PCDD, PCDF, PCB	Storelli et al. (2012)

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treated for conventional (normal cultures) and 5-bromodeoxyuridine (BrdU) incorporation, the latter added 28 h before harvesting at final concentration of 10 μg/ml to obtain preparations to be treated for the SCE-test. Both cell cultures were gently shaken once a day and subjected to 1.5 h of colcemid (0.5 μg/ml) treatment, followed by centrifugation steps, and hypotonic (KCl 75 mM) and fixative treatments according to Iannuzzi and Di Berardino (2008). Slides obtained from both normal and BrdU-treated cultures were stained for 10 min with acridine orange (0.01% in buffer phosphate), washed with distilled water, and mounted in P-buffer. Slides obtained from normal cultures were used to detect structural chromosomal abnormalities (in particular Robertsonian translocation or sex chromosome aneuploidy), slides obtained from

Fig. 1. Localization of the farms investigated: A — control; B — exposed I; C — exposed II; D — exposed III; E — exposed IV; F — exposed V; and G — exposed VI.

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BrdU-treated cells were used for the SCE-test. In particular, slides used for normal cultures were used to get CBA-banding following the protocol reported by Iannuzzi and Di Berardino (2008). At least 20 and 35 complete metaphases (2n = 54) were studied to get CBA-banding and SCE-test, respectively, for each animal. Slides were observed with a NIKON E-1000 fluorescence microscope (Nikon Instruments Europe B.V.) equipped with a FITC specific filter (ex 450–490) and provided with an image-analysis software system (RS Image image-acquisition software, Photometrics Company). Digital images were captured at 100× magnification in gray-scale. All metaphases were carefully examined by at least two expert cytogeneticists.

2.3. Blood redox homeostasis characterization

Plasma concentrations of non-enzymatic antioxidants (Ascorbic Acid, Retinol, and alpha-Tocopherol), and the activities of enzymatic antioxidants (superoxide dismutase and glutathione peroxidase) were measured, and used as markers of the antioxidant defense system. The total antioxidant capacity was also assessed, as well as the oxidative damage to protein and lipid peroxidation induced by the interaction of free radicals with polyunsaturated fatty acids. Therefore, blood samples were collected into heparinized tubes, early in the morning, in the same day and under the same environmental conditions. Plasma was obtained by centrifugation (500 ×g for 15 min at 4 °C) and processed, by the same operator, for titration of antioxidants, SOD and GPx activity, total antioxidant capacity (TAC), nitro-tyrosine (N-Tyr), protein-bound carbonyls (PCs), and hydroperoxides (LPOs).

2.3.1. Determination of antioxidants and lipid hydroperoxides (LPOs)

Plasma samples were processed for determination of Asc concentration as previously described (Spagnuolo et al., 2012), and analyzed by high performance liquid chromatography (HPLC) using an anion exchange column (Nucleosil 100-NH_2, 5 μm, 250 × 4.6 mm i.d). Ret and Toc levels were measured according to a published procedure (Spagnuolo et al., 2003), and analyzed by HPLC, using a reverse phase C18 column (Nova-PAK C18, 4 μm, 125 × 2 mm i.d.). The total antioxidant capacity (TAC) was measured by the Trolox Equivalent Antioxidant Capacity Assay, according to Miller et al. (1993a), and expressed as μM concentration of Trolox equivalents (N.J. Miller et al., 1993; Spagnuolo et al., 2001).

LPO concentration was measured by a colorimetric quantitative assay, using the Lipid Hydroperoxide Assay Kit of Cayman Chemical, according to the manufacturer's instructions.

2.3.2. Determination of nitro-tyrosine (N-Tyr)

Nitrated protein levels in plasma samples were measured by ELISA, as previously reported (Spagnuolo et al., 2001). Plasma samples were diluted (1:500, 1:2000, 1:5000, and 1:10,000) with coating buffer (7 mM Na_2CO_3, 17 mM $NaHCO_3$, 1.5 mM NaN_3, pH 9.6), and incubated in the wells of a microtiter plate overnight at 4 °C. Standard curves were obtained with serial dilutions of nitrated bovine serum albumin (BSA). N-Tyr was detected by incubation with Rabbit anti-N-Tyr antibody (Covalab; 1:800 dilution in 130 mM NaCl, 20 mM Tris–HCl, 0.05% Tween 20, pH 7.3, supplemented with 0.25% BSA; 1 h, 37 °C), followed by Goat anti-Rabbit IgG–horseradish peroxidase linked (GAR–HRP) diluted 1:2000 as the primary antibody. Color development was monitored at 492 nm, as previously described (Spagnuolo et al., 2003). Data were reported as nmol of N-Tyr per mg of protein.

2.3.3. Determination of protein-bound carbonyls (PCs)

PC concentration in plasma samples was titrated by ELISA according to Buss et al. (1997). Protein derivatization was carried out with a dinitrophenylhydrazine (DNP) solution (10 mM in 6 M guanidine hydrochloride, 0.5 M potassium phosphate buffer, pH 2.5), to a final protein concentration of 3 mg/ml. Samples were incubated at room temperature for 45 min vortexing every 10–15 min. Each sample was then diluted (1:800–1:15,000) with 10 mM sodium phosphate buffer, pH 7.0, containing 140 mM NaCl, and incubated (overnight at 4 °C) in the wells of a microtiter plate. PCs were detected by incubation (1 h at 37 °C) with Rabbit anti-DNP antibody diluted 1:1000 with PBS supplemented with 0.2% gelatine and 0.05% Tween 20, followed by GAR–HRP antibody (diluted 1:2500 as the primary antibody). Color development was monitored at 492 nm, as previously described (Spagnuolo et al., 2003). A six-point standard curve of oxidized BSA was included in each plate. A blank for DNP reagent in PBS without protein was subtracted from each absorbance. Data were reported as nmol of carbonyls per mg of proteins.

2.3.4. Evaluation of plasma activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD)

GPx activity was measured indirectly by a coupled reaction with glutathione reductase (GR), using the glutathione peroxidase assay kit of Cayman Chemical, according to the manufacturer's instructions. GPx activity was expressed as nmol of NADPH oxidized per minute per ml of sample.

SOD activity was measured with the superoxide dismutase assay kit of Cayman Chemical, according to the manufacturer's instructions. SOD activity was expressed Unit/ml. One unit of SOD is defined as the amount of the enzyme needed to exhibit 50% dismutation of the superoxide radical.

2.4. Statistical analysis

Summary statistics were calculated for both single animals and animal groups. The distribution of data within each group was tested for normality according to Shapiro and Wilk (1965). Significant departures from symmetry ($P < 0.05$) were observed in all groups with the exception of the exposed VI, whose kurtosis was 0.19. The Log transformation of the data showed that groups with higher SCE means tend also to have more-variable data, indicating that ANOVA on the raw data may be problematic. As consequence, alternative procedures like non-parametric tests are more appropriate. In order to ensure the strictness of the result, both parametric (ANOVA on raw data) and nonparametric tests (Kruskal–Wallis) were performed to point up the differences between and within the analyzed groups. Tukey's and Dwass–Steel–Critchlow–Fligner's pairwise tests were used to make all possible comparisons between the groups. Bonferroni correction was applied as default restriction and differences were considered significant if $P \leq 0.01$.

The samples for measurement of SOD and GPx activities, PC, N-Tyr, or LPO concentration were processed in triplicate. The titration of Toc, Ret, and Asc was carried out in duplicates. Values were expressed as mean ± SD. Significance of statistical differences was evaluated by one-way ANOVA, followed by Bonferroni's test for multiple comparisons, using the Graph Pad Prism 5.01 program (Graph Pad Software, San Diego, CA, USA).

Table 2
Number of animals, examined cells, SCE mean values and standard deviations in sheep reared in polluted and control areas of Sardinia Island (Italy).

Animals		Examined cells		SCEs	
Group	N	N	N	Mean	SD
Exposed I	19	630	5754	8.65[c]	3.40
Exposed II	19	630	5388	8.10[a]	3.50
Exposed III	12	420	3383	8.05[a,b]	3.08
Exposed IV	13	420	3377	7.42[a]	3.34
Exposed V	11	315	3575	9.28[c]	3.56
Exposed VI	18	560	5280	8.38[b,c]	3.29
Control	37	1225	10,185	7.86[a]	3.31

[a,b,c]Means within columns without a common superscript differ ($P < 0.01$).

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Table 3
Parametric (one-way ANOVA) and nonparametric (Kruskal–Wallis H-test) analysis of damaged cells showing differences among treatment groups.

ANOVA						Kruskal–Wallis	
Sources of variation	D. of freedom	Sum of squares	Mean sum of square	*F* ratio		Observed	D. of freedom
Between groups	6	1027.36	171,226	15.45*	H	90.73*	6
Within groups	4193	46,477.4	11.0845				
Total:	4199	47,504.8					

* $P < 0.0001$.

3. Results and discussion

The CBA-banding, very useful to detect sex chromosome abnormalities, did not reveal any numerical and structural abnormality in all studied animals.

The SCE-test was applied to six groups of sheep reared on natural pasturelands near possible polluted areas, as well as on three groups located far from polluted areas and used as control. Analysis of variance using both parametric (ANOVA) and non-parametric (Kruskal–Wallis) approaches revealed significant differences among the groups (Table 3). SCE-mean values per cell were higher in three exposed sheep groups (Esp. I, V and VI) compared to the control. The remaining exposed groups (II, III and IV) did not show significant differences compared to the control (Tables 2 and 4). The mean values of II, III, and IV exposed groups and control were comparable to previously published data on the SCE test for sheep reared in the Campania region (Perucatti et al., 2006). No inter-individual differences were detected among the samples belonging to the same group, whereas inter-group differences were clearly evidenced from multiple comparison tests. In fact, both Tukey's and Dwass–Steel–Critchlow–Fligner's pairwise tests highlighted the same significant differences (Table 4). In particular, the groups I and V showed SCE mean values higher than the number of exchanges counted in the other exposed groups. It seems to be interesting the data of the exposed group III, whose mean SCE value is not different from the control, but also closer to the exposed group VI (Table 2).

Blood redox status of sheep living in different geographic areas of Sardinia, exposed to different environmental contamination, was characterized, and the results were compared with those obtained from animals bred in the unpolluted area. Plasma levels of Ret, Toc, and Asc, the total antioxidant capacity (TAC), and GPx and SOD activities, here used as markers of the antioxidant defense system, are shown in Table 5. The concentrations of Asc, Toc and Ret were found to be significantly lower ($P < 0.001$) in plasma collected from sheep reared in polluted areas (I, II, IV and V) than in the control group. TAC as well as GPx and SOD activities were higher in control than in the exposed groups ($P < 0.001$). These findings demonstrate that the exposure to environmental pollutants severely impairs the blood antioxidant defense system. By comparing the groups from the polluted areas, we found that plasma concentration of Ret and Toc was significantly lower in sheep of group V than in the other exposed groups (I, II and IV; $P < 0.001$), but did not differ among groups from polluted areas (I, II, IV). Conversely, the other markers of the antioxidant defense system did not differ among the two exposed groups. These findings suggest that liposoluble antioxidants play a key role in the protection from environmental pollutants, and that the lipophilic compartment represents a specific target of damage in exposed animals, in agreement with data previously obtained from bovine exposed to steel plant-derived contaminants (Spagnuolo et al., 2012).

The extent of oxidative damage to proteins and lipids was evaluated by measuring plasma concentrations of PC, N-Tyr, and LPO, and a significantly higher extent of oxidative modifications of protein and lipid was also observed in exposed sheep. As shown in Table 6, plasma levels of N-Tyr, PC, and LPO were significantly lower ($P < 0.001$) in the control group than in the exposed groups. In addition, by comparing the four groups from polluted areas, significant differences in the extent of oxidative modifications to plasma proteins and lipids were also found. Indeed, the concentration of N-Tyr, PC and LPO was higher in the plasma from animals of group V ($P < 0.01$ and $P < 0.05$ respectively) than from the other exposed groups. It is worth mentioning that N-Tyr level represents the footprint of protein oxidative damage induced by peroxynitrite (Halliwell, 1997), PC may be introduced into proteins by direct oxidative attack to proteins themselves (Kristal and Yu, 1992), or by reactions with aldehydes originated during lipid peroxidation processes (Uchida and Stadtman, 1993), and LPO is an index of the extent of lipid peroxidation induced by the interaction of free radicals with polyunsaturated fatty acids. Therefore our results strongly suggest that lipid peroxidation and its intermediates, as well as peroxynitrite production, are crucial in determining oxidative modifications to protein and lipid in sheep reared in polluted areas.

On the basis of the location of the sample collection and the results of data analysis, it is clearly shown that differences exist between the groups collected in the northern and southern areas of the island.

Such a result might be connected to the territorial distribution of industrial, military and mining areas. In fact, for its strategic position in the middle-west of the Mediterranean Sea, Sardinia Island has important oil-refineries (for instance Porto Torres) and one of the biggest petrochemical park in Europe (Sarroch industrial estate). Moreover, there is a military training area (Quirra zone) and location of active and disused mines as listed by the Italian Agency for the territory (MATT and APAT, 2006). With the exception of the mining areas spread on the territory, most of the other centers of activity are located in the southern part of the island in a triangle of about 250 km where the groups with higher SCE-means (I, V and VI), lower concentration of Ret and Toc and higher level of N-Tyr, PC and LPO in the plasma (especially V) were sampled. The location of sample collection also explains the trend of the exposed group III which shows intermediate values of SCE. In fact, it is located near a large industrial area for steel production. However, differently from other polluted areas, in this area the values of

Table 4
P-value obtained from multiple comparisons among the groups performed by Tukey pairwise test (above the diagonal) and Dwass–Steel–Critchlow–Fligner pairwise test (below the diagonal). Significant values after Bonferroni correction are indicated by asterisks.

	I	II	III	IV	V	VI	Control
I		0.002*	0.011	<0.0001*	0.146	0.991	<0.0001*
II	0.000*		0.999	0.521	<0.0001*	0.032	0.997
III	0.015	0.986		0.274	<0.0001*	0.099	0.956
IV	<0.0001*	0.564	0.195		<0.0001*	<0.0001*	0.875
V	0.204	<0.0001*	<0.0001*	<0.0001*		0.019	<0.0001*
VI	0.999	0.004*	0.074	<0.0001*	0.096		0.004*
Control	<0.0001*	1	0.934	0.579	<0.0001*	0.000*	

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Table 5
Markers of the antioxidant defense system in plasma of sheep.

	Control	I	II	IV	V
Ret (μg/ml)	0.65 ± 0.05***	0.48 ± 0.04aaa	0.47 ± 0.04ccc	0.46 ± 0.04	0.41 ± 0.03e
Toc (μg/ml)	2.18 ± 0.08***	1.28 ± 0.08aaa	1.26 ± 0.08ccc	1.24 ± 0.05	1.09 ± 0.07eee
Asc (μM)	6.93 ± 0.67***	5.97 ± 0.41bbb	5.37 ± 0.37cc	6.24 ± 0.28ddd	5.85 ± 0.40
GPx (nmol/min/ml)	149.8 ± 46.9***	90.9 ± 30.3	85.5 ± 28.4	101.0 ± 33.6	83.6 ± 27.8
SOD (U/ml)	1.64 ± 0.37***	0.91 ± 0.23	0.86 ± 0.21	1.02 ± 0.25	0.84 ± 0.21
TAC (μM)	106.5 ± 12.3***	86.6 ± 10.0	81.4 ± 9.4	84.8 ± 9.7	78.8 ± 9.1
N	20	20	20	20	20

Ret: Retinol; Toc: α-Tocopherol; Asc: Ascorbate; TAC: total antioxidant capacity (μM concentration of Trolox equivalents); GPx: glutathione peroxidase activity; SOD: superoxide dismutase.
Sheep reared in not polluted area were regarded as control group.
*** Control vs I, II, IV and V ($P < 0.001$).
aaa I vs V ($P < 0.001$).
bbb I vs II ($P < 0.001$).
ccc II vs V ($P < 0.001$).
ddd II vs IV ($P < 0.001$).
eee IV vs V ($P < 0.001$).
e IV vs V ($P < 0.05$).

the contaminants increase when the distance from the industrial pole decreases. This condition might be the result of the wind direction, always reported as E–NE by the Environmental Protection Agency of Sardinia (ARPAS, 2012).

The higher DNA breakages observed in these samples confirm a former study carried out in the Sarroch industrial estate on DNA damages caused by malondialdehyde–deoxyguanosine adducts (Peluso et al., 2013). The data, obtained from this research, show the problematic situation of this region. Indeed, a higher incidence of teratogenesis in animals and a higher cancer incidence for both sheep and sheep farmers of the area V have been reported (Mellis and Lorrai, 2013).

The level of pollution present in these areas was already reported by several studies (Forte et al., 2011; Peluso et al., 2013; Cidu et al., 2013). In particular, among the most represented pollutants there are sulfates, As, Cd, Cr, Hg, Ni, Pb, Zn, Sb, Mn, and Tl, in different distributions in the soil–water–plant system in the south-western part and Rb, Tl, W, Al, and Ti in the PISQ area as reported by ARPAS (2012, 2013). Furthermore, the disused mine sites, in the vicinity of the sample collection sites, still release various metals and metalloids into the environment, contributing negatively to such condition as reported by Varrica et al. (2014).

This mixture of air pollutants has a large impact on people health of this region. In fact, for some combined or mixed exposures the health effects may increase more than the expected effects of a single component (Silins and Högberg, 2011). It is well established that several environmental mutagens may induce chromatin damages (Bryant et al., 2004) and overwhelm antioxidant defenses. Since the chromatin is the main component of chromosomes, damage at the chromosomal level (especially when double DNA breakages occur) may denote chromosome fragility. The higher is this chromosome fragility, the higher is the risk of mutations in animals, and, indirectly humans (Iannuzzi et al., 2004). This enhanced chromosome fragility can result in gene mutations which are often the first step for the onset of cancer, immune-enzymatic defects and reproductive problems. In previous studies, adverse effects of environment air pollution on human and animal reproduction have been demonstrated, including increased risk of fetal growth, women's fertility problems (Dejmek et al., 1999, 2000), decreased sperm quality in young men (Selevan et al., 2000), that included sperm DNA damage, and reduced fertility for the animals (Gustavsson, 1980; Ducos et al., 2008).

Furthermore, since the oxidative stress impairs health, fertility and zootechnical performance of dairy cows (J.K. Miller et al., 1993), and is involved in the etiology of several diseases and metabolic disorders (Smith et al., 1984; Bernabucci et al., 2002, 2005), the exposure to environmental pollutants, as affecting blood redox homeostasis of sheep, might be associated with modifications of physiological and metabolic functions.

This study is a further indication of a potential risk of adverse effect on the health of the exposed population, which suggests the implementation of the surveillance activity in this region, especially in the southern part of the island.

4. Conclusion

To our knowledge, this is the first report of chromosome fragility by a cytogenetic test in livestock reared in Sardinia. The data obtained in this study confirm that animal biomonitoring is a powerful tool for risk assessment from natural and anthropogenic exposure to environmental pollutants. In particular, the monitoring of livestock population by cytogenetic tests might be a good tool to indirectly control the food chain, to preserve health problems, and to avoid management problems and income losses at the farm level. In this respect the herbivores, consuming large volumes of fodder, are the most suitable mammals to

Table 6
Markers of oxidative stress in plasma of sheep. PC: protein-bound carbonyl (nmol per mg of protein); N-Tyr: nitro-tyrosine (nmol per mg of protein); LPO: lipid hydroperoxide (μM).

	Control	I	II	IV	V
PC (nmol/mg P)	18.81 ± 2.87***	47.47 ± 6.68aa	42.72 ± 6.01ccc	38.95 ± 7.38	55.54 ± 7.81eee
N-Tyr (nmol/mg P)	12.58 ± 0.94***	21.93 ± 2.29aaa	19.73 ± 2.06bb, ccc	15.92 ± 0.92ddd	24.12 ± 2.52eee
LPO (μM)	13.37 ± 2.46***	20.46 ± 4.58a	18.55 ± 1.94ccc	17.00 ± 1.89	23.94 ± 5.36eee
N	30	20	20	20	20

Sheep reared in not polluted area were regarded as control group.
*** Control vs I, II, IV and V ($P < 0.001$).
aaa I vs V ($P < 0.001$).
aa I vs V ($P < 0.01$).
a I vs V ($P < 0.05$).
bb I vs II ($P < 0.01$).
ccc II vs V ($P < 0.001$).
ddd II vs IV ($P < 0.001$).
eee IV vs V ($P < 0.001$).

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be used as environmental sentinel. This is particular important in the Sardinia region where the larger Italian sheep population is reared. The SCE data obtained in the present study represent a baseline level for the Sardinian sheep and it represents an essential step for future assessment of health risks in relation to environmental hazards. We also propose that the characterization of blood redox status might represent a useful tool for identifying animals exposed to environmental pollutants. In addition, as plasma concentrations of Ret, Toc, PC, N-Tyr and LPO significantly differ among groups from different geographic areas, with different types and degrees of environmental contamination, they could represent selective and specific markers for bio-monitoring the extent of exposure to specific pollutants.

5. Uncited references

Puggioni, n.d
Siegal and Castellan, 1988

Acknowledgments

The authors wish to thank Ciro Iorio for his technical support in this study. The study has been supported by “Regione Autonoma della Sardegna”, project CRP-24901, L.R. 7/2007.

References

ARPAS, 2012. Agenzia Regionale per la Protezione dell'Ambiente della Sardegna. Piano di disinquinamento per il risanamento del territorio del Sulcis Iglesiente — DPCM 23 aprile 1993. Monitoraggio ambientale anno, pp. 1–80.

ARPAS, Agenzia Regionale per la Protezione dell'Ambiente della Sardegna, 2013. Programma di monitoraggio ambientale del Poligono Interforze del Salto di Quirra (PISQ) stato di avanzamento delle attività dell'ARPAS nella supervisione del programma di monitoraggio ambientale del PISQ. , pp. 1–130 (BOZZA 28/02/2013).

Aumento, F., Le Donne, K., Eroe, K., 2005. Transuranium radionuclide pollution in the waters of the La Maddalena National Marine Park. J. Environ. Radioact. 82, 81–93.

Beccaloni, E., Vanni, F., Beccaloni, M., Carere, M., 2013. Concentrations of arsenic, cadmium, lead and zinc in homegrown vegetables and fruits: estimated intake by population in an industrialized area of Sardinia, Italy. Microchem. J. 107, 190–195.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88, 2017–2026.

Biggeri, A., Lagazio, C., Catelan, D., Pirastu, R., Casson, F., Terracini, B., 2006. Report on health status of residents in areas with industrial, mining or military sites in Sardinia, Italy. Epidemiol. Prev. 30 (1 Suppl. 1), 5–95 (Italian. Erratum in: Epidemiol Prev 2009; 33:75).

Boni, M., Costabile, S., De Vivo, B., Gasparrini, M., 1999. Potential environmental hazard in the mining district of southern Iglesiente (SW Sardinia, Italy). J. Geochem. Explor. 67, 417–430.

Bryant, P.E., Gray, L.J., Peresse, N., 2004. Progress towards understanding the nature of chromatid breakage. Cytogenet. Genome Res. 104, 65–71.

Budroni, M., Sechi, O., Cossu, A., Palmieri, G., Tanda, F., Foschi, R., Rossi, S., 2013. Estimates of cancer burden in Sardinia. Tumori 99, 408–415.

Buss, H., Chan, T.P., Sluis, K.B., Domigan, N.M., Winterbourn, C.C., 1997. Protein carbonyl measurement by a sensitive ELISA method. Free Radic. Biol. Med. 23, 361–366.

Cadenas, E., Davies, K.J., 2000. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic. Biol. Med. 29, 222–230.

Castillo, C., Hernández, J., López Alonso, M., Miranda, M., Benedito, J.L., 2003. Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. Archiv. Tierzucht. 46, 227–233.

Castillo, C., Hernández, J., Bravo, A., López Alonso, M., Pereira, V., Benedito, J.L., 2005. Oxidative status during late pregnancy and early lactation in dairy cows. Vet. J. 169, 286–292.

Castillo, C., Hernández, J., Valverde, I., Pereira, V., Sotillo, J., López Alonso, M., Benedito, J.L., 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res. Vet. Sci. 80, 133–139.

Castillo, C., Hernández, J., García Vaquero, M., López Alonso, M., Pereira, V., Miranda, M., Blanco, I., Benedito, J.L., 2012. Effect of moderate Cu supplementation on serum metabolites, enzymes and redox state in feedlot calves. Res. Vet. Sci. 93, 269–274.

Cavallo, D., Ursini, C.L., Maiello, R., Apostoli, P., Catalani, S., Ciervo, A., et al., 2008. Genotoxic and oxidative effects induced on A549 cells by extract of PM_{10} collected in an electric steel plant. Acta Biomed. 7, 87–96.

Cidu, R., Biddau, R., Dore, E., Vacca, A., 2013. Antimony dispersion at abandoned mines in Sardinia, Italy. Procedia Earth Planet. Sci. 7, 171–174.

Cidu, R., Biddau, R., Dore, E., Vacca, A., Marini, L., 2014. Antimony in the soil–water–plant system at the Su Suergiu abandoned mine (Sardinia, Italy): strategies to mitigate contamination. Sci. Total Environ. 16 (497–498C), 319–331.

Cristaldi, M., Foschi, C., Szpunar, G., Brini, C., Marinelli, F., Triolo, L., 2013. Toxic emissions from a military test site in the territory of Sardinia, Italy. Int. J. Environ. Res. Public Health 10, 1631–1646.

De Luca, G., Furesi, A., Leardi, R., Micera, G., Panzanelli, A., Piu, P.C., Sanna, G., 2004. Polycyclic aromatic hydrocarbons assessment in the sediments of the Porto Torres Harbor (Northern Sardinia, Italy). Mar. Chem. 86, 15–32.

Dejmek, J., Selevan, S.G., Benes, I., Solanský, I., Srám, R.J., 1999. Fetal growth and maternal exposure to particulate matter during pregnancy. Environ. Health Perspect. 107, 475–480.

Dejmek, J., Solanský, I., Benes, I., Lenícek, J., Srám, R.J., 2000. The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. Environ. Health Perspect. 108, 1159–1164.

Di Meo, G.P., Perucatti, A., Fornataro, D., Incarnato, D., Ferrara, L., Matassino, D., Iannuzzi, L., 2000. Sister chromatid exchange in chromosomes of sheep (*Ovis aries*). Cytobios 101, 71–78.

Di Meo, G.P., Perucatti, A., Genualdo, V., Caputi-Jambrenghi, A., Rasero, R., Nebbia, C., Iannuzzi, L., 2011. Chromosome fragility in dairy cows exposed to dioxins and dioxin-like PCBs. Mutagenesis 26, 269–272.

Ducos, A., Revay, T., Kovacs, A., Hidas, A., Pinton, A., Bonnet-Garnier, A., Molteni, L., Slota, E., Switonski, M., Arruga, M.V., van Haeringen, W.A., Nicolae, I., Chaves, R., Guedes-Pinto, H., Andersson, M., Iannuzzi, L., 2008. Cytogenetic screening of livestock populations in Europe: an overview. Cytogenet. Genome Res. 120, 26–41.

Forte, G., Madeddu, R., Tolu, P., Asara, Y., Marchal, J.A., Bocca, B., 2011. Reference intervals for blood Cd and Pb in the general population of Sardinia (Italy). Int. J. Hyg. Environ. Health 214, 102–109.

Gatti, A.M., Montanari, S., Capitani, F., 2013. The Quirra syndrome: matter of translational medicine. NATO Science for Peace and Security Series B: Physics and Biophysics pp. 55–64.

Genualdo, V., Perucatti, A., Iannuzzi, A., Di Meo, G.P., Spagnuolo, S.M., Caputi-Jambrenghi, A., Coletta, A., Vonghia, G., Iannuzzi, L., 2012. Chromosome fragility in river buffalo cows exposed to dioxins. J. Appl. Genet. 53, 221–226.

Goulart, M., Batoréu, M.C., Rodrigues, A.S., Laires, A., Rueff, J., 2005. Lipoperoxidation products and thiol antioxidants in chromium exposed workers. Mutagenesis 20, 311–315.

Gustavsson, I., 1980. Chromosome aberrations and their influence on the reproductive performance of domestic animals — a review. Z. Tierzucht. Zuchtgsbio 97, 176–195.

Halliwell, B., 1997. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo? FEBS Lett. 411, 157–160.

Halliwell, B., 2012. Free radicals and antioxidants: updating a personal view. Nutr. Rev. 70, 257–265.

Halliwell, B., Gutteridge, J.M.C., 2000. Free radicals, other reactive species and disease. In: Halliwell, B., Gutteridge, J.M.C. (Eds.), Free Radicals in Biology and Medicine. Oxford University Press, Oxford, UK, pp. 617–783.

Hernandez, R.Y.S., Zucchetti, M., Aumento, F., Gual, M.R., Cozzella, M.L., Hernandez, C.M.A., 2011. Measurement of plutonium pollution in sediments and algae in marine environment: Cienfuegos Bay and La Maddalena Islands. Fresenius Environ. Bull. 20, 802–809.

Iannuzzi, L., Di Berardino, D., 2008. Tools of the trade: diagnostics and research in domestic animal cytogenetics. J. Appl. Genet. 49, 357–366.

Iannuzzi, L., Perucatti, A., Di Meo, G.P., Polimeno, F., Ciotola, F., Incarnato, D., Peretti, V., Caputi-Jambrenghi, A., Pecoraro, A., Manniti, F., D'Alessandro, A., Vonghia, G., 2004. Chromosome fragility in two sheep flocks exposed to dioxins during pasturage. Mutagenesis 19, 355–359.

Ishida, T., Takeda, T., Koga, T., Yahata, M., Ike, A., Kuramoto, C., et al., 2009. Attenuation of 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity by resveratrol: a comparative study with different routes of administration. Biol. Pharm. Bull. 32, 876–881.

Knerr, S., Schaefer, J., Both, S., Mally, A., Dekant, W., Schrenk, D., 2006. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induced cytochrome P450s alter the formation of reactive oxygen species in liver cells. Mol. Nutr. Food Res. 50, 378–384.

Kristal, B.S., Yu, B.P., 1992. An emerging hypothesis: synergistic induction of aging by free radicals and Maillard reactions. J. Gerontol. 47, B107–B114.

Madeddu, R., Tolu, P., Asara, Y., Farace, C., Forte, G., Bocca, B., 2013. Blood biomonitoring of metals in subjects living near abandoned mining and active industrial areas. Environ. Monit. Assess. 185, 5837–5846.

Matés, J.M., Segura, J.A., Alonso, F.J., Marquez, J., 2010. Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms. Free Radic. Biol. Med. 49, 1328–1341.

Mateuca, R., Aka, P.V., De Boeck, M., Hauspie, R., Kirsch-Volders, M., Lison, D., 2005. Influence of hOGG1, XRCC1 and XRCC3 genotypes on biomarkers of genotoxicity in workers exposed to cobalt or hard metal dusts. Toxicol. Lett. 1562, 277–288.

MATT, Ministero dell'Ambiente e della Tutela del Territorio, APAT, Agenzia per la Protezione dell'Ambiente e per i Servizi Tecnici, Aprile 2006. Censimento dei siti minerari abbandonati. 1–95.

Mellis, G., Lorrai, S., 2013. Monitoraggio ambientale al PISQ. Available online:. http://www.fnovi.it/index.php?pagina=pubblicazioni.

Miller, N.J., Rice-Evans, C.A., Davies, M.J., Gopinathan, V., Milner, A., 1993a. A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates. Clin. Sci. (Lond.) 84, 407–412.

Miller, J.K., Brzezinska-Slebodzinska, E., Madsen, F.C., 1993b. Oxidative stress, antioxidants, and animal function. J. Dairy Sci. 76, 2812–2823.

Peluso, M., Munnia, A., Ceppi, M., Giese, R.W., Catelan, D., Rusconi, F., Godschalk, R.W., Biggeri, A., 2013. Malondialdehyde–deoxyguanosine and bulky DNA adducts in schoolchildren resident in the proximity of the Sarroch industrial estate on Sardinia Island, Italy. Mutagenesis 28, 315–321.

Penders, E.J., Spenkelink, A., Hoogenboezem, W., Rotteveel, S.G., Maas, J.L., Alink, G.M., 2012. Genotoxic effects in the Eastern mudminnow (*Umbra pygmaea*) after prolonged exposure to River Rhine water, as assessed by use of the in vivo SCE and Comet assays. Environ. Mol. Mutagen. 53, 304–310.

Perucatti, A., Di Meo, G.P., Albarella, S., Ciotola, F., Incarnato, D., Jambrenghi, A.C., Peretti, V., Vonghia, G., Iannuzzi, L., 2006. Increased frequencies of both chromosome

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abnormalities and SCEs in two sheep flocks exposed to high dioxin levels during pasturage. Mutagenesis 21, 67–75.
Pirastu, R., Zona, A., Ancona, C., Bruno, C., Fano, V., Fazzo, L., Iavarone, I., Minichilli, F., Mitis, F., et al., 2011. Risultati dell'analisi della mortalità nel Progetto SENTIERI (Mortality results in SENTIERI Project). Epidemiol. Prev. 35, 29–152.
Q31 Puggioni, A. Aspetti geografici e normativi delle bonifiche delle aree inquinate del nord Sardegna. Università degli Studi di Sassari. Dipartimento di Scienze Umanistiche e Sociali. Dottorato di Ricerca in Scienze dei Sistemi Culturali. XXVI ciclo.
Rubes, J., Pokorná, Z., Borkovec, L., Urbanová, J., Strnadová, V., 1997. Dairy cattle as a bioindicator of exposure to genotoxic substances in a heavily polluted area in northern Bohemia. Mutat. Res. 391, 57–70.
Safronova, V.I., Piluzza, G., Zinovkina, N.Y., Kimeklis, A.K., Belimov, A.A., Bullitta, S., 2012. Relationships between pasture legumes, rhizobacteria and nodule bacteria in heavy metal polluted mine waste of SW Sardinia. Symbiosis 58, 149–159.
Sanna, E., Liguori, A., Palmas, L., Soro, M.R., Floris, G., 2003. Blood and hair lead levels in boys and girls living in two Sardinian towns at different risks of lead pollution. Ecotoxicol. Environ. Saf. 55, 293–299.
Schintu, M., Degetto, S., 1999. Sedimentary records of heavy metals in the industrial harbour of Portovesme, Sardinia — Italy. Sci. Total Environ. 241, 129–141.
Selevan, S.G., Borkovec, L., Slott, V.L., Zudová, Z., Rubes, J., Evenson, D.P., Perreault, S.D., 2000. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ. Health Perspect. 108, 887–894.
Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52, 591–661.
Siegal, S., Castellan Jr., N.J., 1988. Nonparametric Statistics for the Behavioral Sciences. 2nd. McGraw-Hill, New York (Jr 399 pp.).
Silins, I., Högberg, J., 2011. Combined toxic exposures and human health: biomarkers of exposure and effect. Int. J. Environ. Res. Public Health 8, 629–647.
Spagnuolo, M.S., Cigliano, L., Balestrieri, M., Porta, A., Abrescia, P., 2001. Synthesis of ascorbate and urate in the ovary of water buffalo. Free Radic. Res. 35, 233–243.
Spagnuolo, M.S., Cigliano, L., Sarubbi, F., Polimeno, F., Ferrara, L., Bertoni, G., et al., 2003. The accumulation of alpha-Tocopherol and Retinol in the milk of water buffalo is correlated with the plasma levels of triiodothyronine. Biofactors 19, 197–209.
Spagnuolo, M.S., Cigliano, L., Nebbia, C., Rossetti, C., Grazioli, G., Iannuzzi, L., 2012. Analysis of plasma indices of redox homeostasis in dairy cows reared in polluted areas of Piedmont (northern Italy). Sci. Total Environ. 433, 450–455.
Storelli, M.M., Scarano, C., Spanu, C., De Santis, E.P.L., Busco, V.P., Storelli, A., Marcotrigiano, G.O., 2012. Levels and congener profiles of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in sheep milk from an industrialised area of Sardinia, Italy. Food Chem. Toxicol. 50, 1413–1417.
Uchida, K., Stadtman, E.R., 1993. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction. J. Biol. Chem. 268, 6388–6393.
van der Schalie, W.H., Gardner Jr., H.S., Bantle, J.A., De Rosa, C.T., Finch, R.A., Reif, J.S., Reuter, R.H., Backer, L.C., Burger, J., Folmar, L.C., Stokes, W.S., 1999. Animals as sentinels of human health hazards of environmental chemicals. Environ. Health. Perspect. 107, 309–315.
Varrica, D., Tamburo, E., Milia, N., Vallascas, E., Cortimiglia, V., De Giudici, G., Dongarrà, G., Sanna, E., Monna, F., Losno, R., 2014. Metals and metalloids in hair samples of children living near the abandoned mine sites of Sulcis–Inglesiente (Sardinia, Italy). Environ. Res. 8 (134C), 366–374.
Wójcik, E., Smalec, E., 2013. The effect of environmental factors on sister chromatid exchange incidence in domestic horse (*Equus caballus*) chromosomes. Folia Biol. (Krakow) 61, 199–204.
Yang, H.Y., Liu, J., Yang, S.Y., Wang, H.Y., Wang, Y.D., 2014. Increased sister chromatid exchange in peripheral blood lymphocytes from humans exposed to pesticide: evidence based on a meta-analysis. Asian Pac. J. Cancer Prev. 15, 9725–9730.

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