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Micronucleus Frequency in Cytokinesis-Blocked Bovine Lymphocytes from Regions with Different Pollution Levels in Slovakia

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Animal breeding can be affected by genotoxic agents from industrial pollution as well as the excessive use of chemicals in agriculture. Exposure to genotoxic agents may result in mutations, metabolic disorders, reduced fertility or immunosuppression. Biological monitoring of farm animal populations is important in order to preserve the health of animals, to control the quality of foods of animal origin and to assess the genotoxic effects of factors influencing human health (Rubeš et al., 1992; Parada and Jaszczak, 1993; Di Berardino et al., 1997).

Cytogenetic biomarkers, chromosome aberrations, SCE and the micronucleus test play an important role in animal monitoring studies (Hebert and Murdoch, 1996; Lessire et al., 1997). The micronucleus test on lymphocytes of mammalian species as performed by Fenech and Morley (1985) appears to be a simple, rapid and inexpenxive test for in vitro or in vivo evaluation of the effects of genotoxic treatment. The expression of micronuclei may be the consequence of chromosome aberrations or spindle dysfunction, consequently the micronucleus test can be used to detect both clastogens and aneuploidogens (Norppa et al., 1993; Fenech, 1993).

In nine regions of the Slovak Republic the quality of the elementary components of environment is rather impaired. Central Spiš, Central Gemer, Košice and Central Zemplín appear to be the most affected regions of Eastern Slovakia. In this study the micronucleus test was used to monitor the genotoxic effects of pollutants in bovine lymphocytes obtained from animals reared on farms in the Košice and the Central Zemplín regions as well as on one ecological farm.

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MATERIALS AND METHODS

In the spring of 1998, blood samples were obtained from dairy cattle from three farms. On the ecological farm (Farm I) ten 3 - 5-year-old Slovak Pinzgau cattle were examined. On Farm II, situated in the Košice region 2.5 km away from the East Slovakian Iron Works which pollute the environment with SO_2, NO_x, CO, Fe, Zn, Mn, Cu, As, Cd and Pb emissions, samples were taken from ten 3 - 4-year-old and two 6-year-old Holstein-Friesian cows. On Farm III, situated in the Central Zemplín region where chemical enterprises contaminate the environment with SO_2, NO_x, Cd, carbohydrates, hydrosulphide, chlorine, mercaptans and a mixture of organic compounds and dust (Pavlík et al., 1997), twenty 3 - 5-year-old Pinzgau cows were sampled (see Figure 1).

Whole blood samples (0.4 mL) were cultured at 37.5 °C in 7.0 mL S-Chromo-Cell chromosome medium supplemented with FCS, PHA-L, L-Glutamin (PAN Systems GmbH Biotechnologische Produkte, Germany), antibiotics (Penicillin G 100 IU/mL and Streptomycin, 100 μg/mL) and 7.5 % $NaHCO_3$ (Sevac, a.s., Czech Republic). The cultures were harvested after 72 hours of growth using standard cytogenetic methods.

Cytokinesis-blocked micronucleus assay: After 44 hours of incubation 2 mg/mL cytochalasin B (Sigma, St. Luis, USA) dissolved in DMSO were added to give a final concentration of 6 μg/mL and the cultures were incubated for another 28 hours. The cells were collected by centrifugation, resuspended in hypotonic solution at 37.5 °C (0.055 M KCl) for 20 min. and fixed in 3:1 (v/v) methanol:acetic acid mixture. The preparations were air-dried and the slides stained with 10% Giemsa solution in Sörensen phosphate buffer at pH 6.8 for 7 minutes. Using a Nikon microscope the coded slides were scored blindly at a magnification of 400x. Micronuclei (MN) were identified applying well-known criteria (Countryman and Heddle, 1976; Surrallés and Natarajan, 1997). In order to evaluate MN frequency 1000 binucleated cells (BC) were examined on slides coded separately for each animal.
Statistical methods: The distribution of BC with MN and the total number of MN on the individual farms were compared. One-way analysis of variance (ANOVA) was used. Assessment of differences between the farms was performed by the Tukey test. For data analysis the Sigma Stat statistical software (Jandel Scientific®) was used.

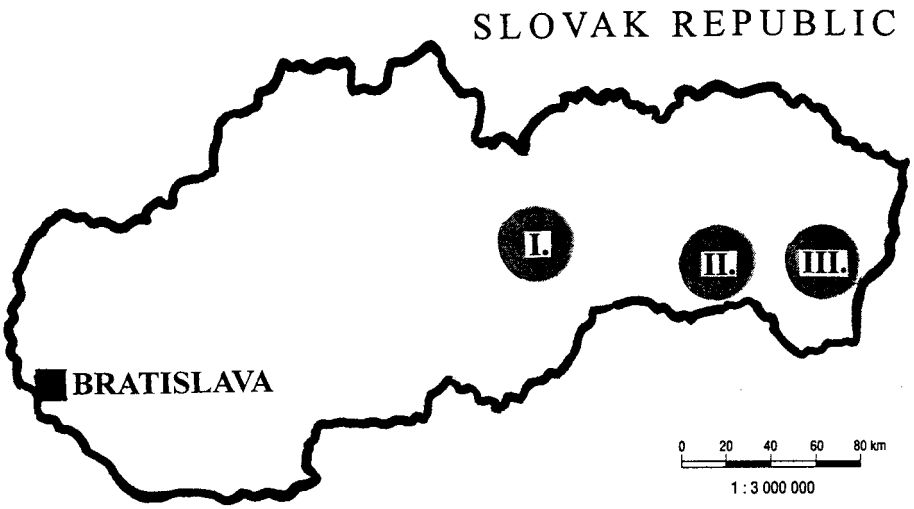

Figure 1. Map of Slovakia showing the regions of the study. I. - ecological farm, II. - Košice region, III. - Central Zemplín region.

RESULTS AND DISCUSSION

The results of the micronucleus test performed in lymphocytes of dairy cows from regions with uneven pollution of the environment are given in table 1. The lowest frequency of micronuclei was observed in the samples from Farm I (23.20 ± 8.82 MN per 1000 BC). In samples from the polluted regions (Farms II and III), 26.17 ± 9.17 and 37.50 ± 13.78 MN were counted per 1000 BC, respectively.

When comparing micronucleus frequency on Farm III to that on Farms I and II, respectively, significant differences were recorded that each time disadvantaged Farm III (see Figure 2). Monitoring of chromosome aberrations in farm animals is helpful when assessing the hygienic level in herds possibly exposed to genotoxicants (Rubeš et al., 1992). It is widely accepted that the occurrence of micronuclei in proliferating mammalian cells provides unequivocal evidence of cytogenetic impairment resulting from broken or lagging chromosomes (Fenech, 1993). Scarfi et al. (1993) were the first to report on the frequency of micronuclei in the lymphocytes of Fresian cattle in Italy (12.30 ± 4.40 per 500 BC), however, without mentioning the age of the animals although age is an important factor in micronucleus frequency (Peace and Succop, 1999). The results by Scarfi et al. agree with the findings of micronuclei in our samples from the ecological farm.

Table 1. Micronucleus frequency in binucleated cells of bovine lymphocytes from different regions.

Subject	Sex	MN frequencies per thousand binucleated cells		
		Ecological farm I.	Košice region II.	Middle Zemplín region III.
1.	F	23	23	28
2.	F	17	18	48
3.	F	15	19	48
4.	F	25	19	44
5.	F	23	23	35
6.	F	34	35	40
7.	F	28	37	28
8.	F	18	22	30
9.	F	39	42	28
10.	F	10	26	44
11.	F		30	31
12.	F		30	30
13.	F			15
14.	F			63
15.	F			23
16.	F			22
17.	F			43
18.	F			72
19.	F			43
20.	F			35
	MEAN	23.20	26.17	37.50
	(Std Dev)	8.82	9.17	13.78
	(SEM)	2.79	2.65	3.08

The differences in the mean values among treatment group are greater than would be expected by chance, there is a statistically significant difference ($P = 0.004$). StD Dev: Standard Deviation, SEM: Standard Error Mean.

Buleca et al. (1998) studied the effects of genotoxic pollutants of the environment on the frequency of micronuclei in the lymphocytes of twelve 3 - 8-year-old dairy cows from Farm Grajciar in the Košice region. The analyses were performed in the autumn; they revealed 23.23 ± 6.36 MN per 1000 BC and proved to be insignificant at the level of $P > 0.05$ when compared to the controls (21.00 ±5.43 MN per 1000 BC).

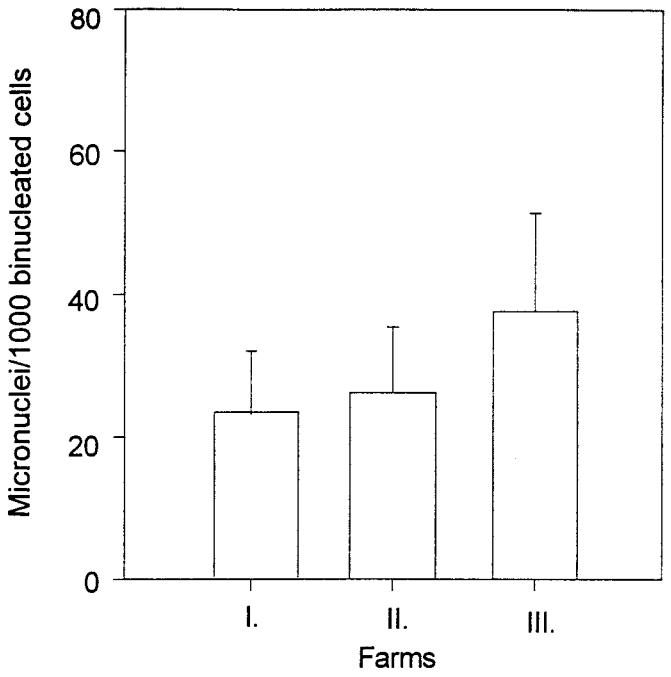

Figure 2. Bart chart column means:
All pairwise multiple comparison procedures (Tukey Test).
Comparisons for factor:

Diff. of Means	p	q	P<0.05
III. vs. I. 14.300	3	4.508	yes
III. vs. II. 11.333	3	3.790	yes
II. vs. I. 2.967	3	0.846	no

Increased micronucleus frequency may point at environment-induced genetic damage to the organism. In 1996 and 1997 Danielovič (1998) recorded polychlorinated biphenyl levels (congeners no. 28 and 52) in the soil of Central Zemplin localities that surpassed the limits several times. The frequency of micronuclei in the lymphocytes of dairy cows from the Košice and Central Zemplín regions coincides with the frequency of chromosome aberrations simultaneously observed by Šutiaková et al. (2000) in the same localities (2.75 ± 2.25 and 4.00 ± 3.01 % of aberrant cells, respectively).

Our results revealed an increased frequency of micronuclei in binucleated cells. Since industrial pollutants may alter the stability of the genome and thus present a potential genetic hazard, biological screening of the macro- and microclimate in different animal species is of great importance.

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