

THE FREQUENCY OF MICRONUCLEI IN THE PERIPHERAL LYMPHOCYTES OF SHEEP IN CONVENTIONAL AND ECOLOGICAL FARMING

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Summary

In this work the occurrence of micronuclei in the peripheral lymphocytes during the autumn period was studied in ecologically and conventionally farmed Improved Wallachian sheep. In the above groups of 1-4-year-old sheep a frequency of 14.4 ± 6.13 and 17.5 ± 14.01 micronuclei per 1000 binucleated cells was observed, respectively. In rams, 34.11 ± 14.62 and 20.33 ± 6.44 micronuclei were stated for the conventional and ecological farm, respectively, the results revealing significance at the level of $P = 0.02$.

Key words: genotoxins, frequency of micronuclei, sheep, ecological and conventional farming

Farm animals are exposed to different substances present in soil, water, air or feeds. The biological effects of these substances, i.e., the response of the organism to the impact of genotoxic substances in different ecosystem can be assessed by different cellular, histological, biochemical and cytogenetic biomarkers (Schramm et al., 1999; De Souza Bueno, 2000). Chromosome aberrations, sister exchange chromatids and micronuclei as cytological biomarkers are used in biomonitoring studies of animals exposed to environmental genotoxins (Hebert, Luiker, 1996). According to Di Bernardino et al. (1997), environmental genotoxin monitoring with cytogenetic biomarkers is especially important with respect to bioproducts since farm areas are polluted with pesticides, mycotoxins, heavy metals and the like.

Breeding animals under specific conditions are an integrated part of the ecological farming system (Fazekašová, Poráčová, 1999). Kováč (1996) pointed at differences in animal breeding in the conventional and ecological farming systems.

This work aimed at assessing the occurrence of micronuclei in the peripheral lymphocytes of sheep under conventional and ecological farming practices.

Materials and methods

In the autumn, Improved Wallachian sheep aged 1 – 4 years (10 males, 9 females) were examined on an ecologically operating farm (Anonymous, 1995). Sheep of the same breed aged 2 – 5 years and coming from a conventional farm (9 males, 8 females) were also subjected to analysis.

Biological material – blood – was obtained from the v. jugularis into heparin (100 IU/ml of blood). Heparinized blood (0.4 ml) was cultured in 0.6 ml of Panserin 702-Chromosome Medium-S-chromo-cell with FBS, PHA-L and L-glutamine (PAN Systems GmbH, Biotechnologische Produkte TM, Germany) for 72 hours at 37.5 °C. Antibiotics (100 IU/ml penicillin G and 100 µg/ml streptomycine) and 7.5% NaHCO₃ were added to the culturing medium.

Micronucleus test: After 44 h of culturing cytochalasin B (2 mg/ml in DMSO, Sigma, USA) was added to the medium at a final concentration of 6 µg/ml. The preparations were evaluated in a Nikon microscope using Animal and Photostyler software at a magnification of 400x and 1000x. Micronuclei were identified according to the criteria of Countryman and Heddle (1976). The frequency of micronuclei was determined per 1000 binucleated cells for each animal. The results were statistically evaluated using the Sigma stat (Jandel Scientific[®]) t-test.

Table 1 Frequency of micronuclei in the peripheral lymphocytes of ecologically and conventionally farmed sheep in the autumn period

Donor Sex	Statistical values	MN frequency per 1000 cells	
		Conventional farm	Ecological farm
F	x —	17,5	14,4
n=8	Std Dev	14,01	6,13
n=10 (E)	SEM	4,95	1,94
	P=0,05		
M	x —	34,11	20,33
n=9	Std Dev	14,62	6,44
n=9(E)	SEM	4,88	2,15
	P=0,02		

Note: If more micronuclei occurred wicch binucleated cells, their number was included in the total. F =female, M=male, E=ecological farm.

Results

In autumn, the peripheral lymphocytes of female sheep in the conventional and ecological system revealed a frequency of 17.5 ± 14.01 and 14.4 ± 6.13 micronuclei per 1000 binucleated cells, respectively, however, without statistical significance ($P = 0.05$; Table 1).

As can be seen from the table, significant results ($P = 0.02$) concerning micronucleus frequency in the peripheral lymphocytes were obtained in the male sheep of both systems.

Discussion

The micronucleus test is used in biomonitoring studies in both human and veterinary medicine dealing with environmental load (Dianovský, 1994; Kovalkovičová et al., 2001; Šutiaková et al., 2001; Bonassi et al., 2001). According to Fenech (1998) the frequency of micronuclei can be affected by several factors (age, life style, sex, individual mutagen sensitivity, etc.) which therefore need to be studied step by step. In earlier studies the spontaneous frequency of micronuclei in the lymphocytes of conventionally and ecologically bred sheep was assessed during the spring period (Šutiaková et al., 2000). Since these cytogenetic analyses characterized the response of the organism to genotoxins of the preceding period of 3-4 months, we took interest in the factor of seasonality. In autumn, the frequency of micronuclei in the peripheral lymphocytes of females presented 17.5 ± 14.1 and 14.4 ± 6.13 per 1000 binucleated cells in the conventional and ecological system, respectively. Similar results were obtained in spring, however, on the conventional farm both sexes were taken into account for frequency assessment. In the lymphocytes of rams a frequency of 34.11 ± 14.62 and 20.33 ± 6.44 micronuclei per 1000 binucleated cells was stated on the conventional and the ecological farm, respectively, These figures revealed significance. In connection with the before said a specific feature of sheep breeding has to be pointed out: breeding rams usually come from other localities (market purchase) then the ewes under investigation and thus the results may be „distorted,“ (Kvokačka, personal communication). This fact needs to be recalled when considering the high frequency of micronuclei (60 – 70 per 1000 binucleated cells) in two of the breeding rams. In peripheral lymphocytes of dairy cows Scarfi et al. (1993) reported a spontaneous frequency of 12.3 ± 4.1 micronuclei per 500 binucleated cells and observed it to be 3x higher than in man. This fact was attributed to sensitivity to cytochalasin B, the number of chromosomes in cattle ($2n = 60$) and sensitivity to genotoxic pollutants.

Vith view to the continuing presence of different genotoxic substances in the environment the importance of using cytogenetic biomarkers to prevent health disturbances and produce ecological food is still increasing.

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