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USE OF THE MICRONUCLEUS TEST IN ASSESSING THE GENOTOXICITY OF THE ENVIRONMENT IN PIGGERIES

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ABSTRACT

Pigs as a species are very susceptible to impaired life conditions. The micronucleus test enables to state chromosomal damage caused by genotoxic substances in the environment in which the organism is living. The bioclimatic indices in the environment of the piggery under examination were proved to be appropriate. The frequency of micronuclei in boars reached 9.3 $\pm$ 2.49 per 1000 binucleated cells whereas in sows prior to and after delivery 6.98 $\pm$ 4.75 and 6.82 $\pm$ 2.66 micronuclei were stated per 1000 binucleated cells, respectively. Irrespective of sex the frequency of micronuclei in pigs reached 7.7 $\pm$ 3.45 per 1000 binucleated cells whereas in sows at different stages of the reproductive cycle 6.9 $\pm$ 3.7 micronuclei were counted per 1000 binucleated cells.

Key words: pigs, genotoxicity of the environment, micronucleus test

INTRODUCTION

Environmental genotoxic substances found in the macro- and microclimate affect not only the health state of farm animals but also their production indices and present a load for the human food chain (Parada, Jaszczak, 1993; Hebert, Luiker, 1996). For this reason attention is paid to the prevention of health disturbances in animals living in different ecosystems using suitable biomarkers and bioindicators (Lessire et al., 1997). Pigs as a species are very susceptible to unsuitable life conditions. According to Donhan (2000) chemical, biological, infectious, biomechanical, thermal and other physical factors present the greatest risks to the overall health of pigs. Different monitoring systems have been developed which use the above aspects as indicators of the potential risks to animal health. The micronucleus test is a means for the determination of chromosomal damage caused by the genotoxic substances to which the organism is exposed in the environment (Bonassi et al. 2001a). Micronuclei develop from acentric chromosome fragments or whole chromosomes which had not been incorporated in the nucleus during mitosis in the course of cell division (Heddle et al. 1991).

It was the aim of this work to determine the frequency of micronuclei in the peripheral lymphocytes of sows prior to and after delivery as well as in boars.

MATERIALS AND METHODS

Animals: In spring, 6 boars and 12 sows of the White Improved, Landrace and Yorkshire breeds aged 1-3 years were examined on the farm K.P. The animals were housed in mass

stables divided by manipulation corridors into four compartments. Each compartment comprised a dunging yard covered with slats and a concrete resting area . In the latter the feeding and watering technology was placed as part of a stationary feeding line. The bioclimatic regime in the stables depended on the temperature dynamics of the outer environment, i.e., it was strongly season-dependent.

Biological material: For chromosome analysis blood samples were taken from the *v. auricularis* into heparin (500 IU/ml blood). The samples (0.4 ml) were cultured at 37.5 °C in 7.0 ml S-Chromo-Cell Chromosome medium (PAN Systems GmbH Biotechnologische Produkte, Germany ™) supplemented with FCS, PHA-L and L-glutamin, 100 IU/ml penicillin G, 100 µg/ml streptomycin and 7.5% $NaHCO_3$.

Micronucleus test: Culturing was carried out for 72 hours; after 44 h cytochalasin B (Sigma, USA) was added at a final concentration of 6 µg/ml.

The samples were evaluated in a Nikon microscope using Animal and Photostyler software at a magnification of 400x and 1000x. The micronuclei were identified according to the criteria of Countryman and Heddle (1976). The frequency of micronuclei per 1000 binucleated cells was determined for each animal. The results were statistically processed with the Sigma Stat^R programme (ANOVA, equal variance test).

RESULTS

In the spring period the frequency of micronuclei in the peripheral lymphocytes of boars was 9.3 ± 2.49 / 1000 binucleated cells whereas in the sows prior to and after delivery frequencies of 6.98 ± 4.75 and 6.82 ± 2.66 micronuclei were observed per 1000 binucleated cells. As can be seen from Table 1, no significant differences were stated between the groups under observation. In sows 6.9 ± 3.7 micronuclei were stated per 1000 binucleated cells at different reproduction periods whereas in pigs irrespective of their sex 7.7 ± 3.45 micronuclei were found per 1000 binucleated cells, but without significant changes.

DISCUSSION

Since farm areas are often polluted with pesticides, mycotoxins, heavy metals etc., monitoring of environmental mutagens by cytogenetic biomarkers is of special importance for the quality of animal products (Di Berardino et al., 1997). Rubeš et al. (1988) studied the frequency of chromosome aberrations and SCE in the peripheral lymphocytes of swine in 5 piggeries; simultaneously these authors analyzed Zn, Pb, Cd, Hg, aflatoxin, PCB, DDT and lindan levels in the stables. They observed an increased frequency of aberrant cells on some of the investigated farms and noticed that these biomarkers could be used at the hygienic control of the level of exposition of pigs to mutagens. Our investigation was concerned with a cytogenetic biomarker - the micronucleus test - since it is simple, reliable and rapid. In the peripheral lymphocytes of boars as well as of sows in different phases of the reproduction process 9.3 ± 2.49 and 6.9 ± 3.7 micronuclei were counted per 1000 binucleated cells, respectively. There was no possibility to compare the results of this investigation with the data of other authors. The results show that there is a considerable difference between the individual animals therefore a greater number of animals needs to be analyzed in relation to a defined bioclimate and health state. Our results also point at the fact that determination of the

frequency of micronuclei is of great importance in the prevention of health disturbances as well as in the ecological production of food. From the environmental viewpoint ecological farms fulfill certain special requirements in relation to primary agricultural production (Bröcker, 1998). Biomarkers should serve as early indicators of health risks (Bonassi et al., 2001b).

CONCLUSION

This research was aimed at the study of the frequency of micronuclei in the peripheral lymphocytes of boars and sows at different stages of the reproduction cycle. The physical factors of the bioclimate in the selected piggery proved to be suitable. The frequency of micronuclei in boars reached 9.3 $\pm$ 2.49 per 1000 binucleated cells whereas in sows prior to and after delivery 6.98 $\pm$ 4.75 and 6.82 $\pm$ 2.66 micronuclei were stated per 1000 binucleated cells, respectively. Irrespective of sex the frequency of micronuclei in pigs reached 7.7 $\pm$ 3.45 per 1000 binucleated cells.

Table 1: Frequency of micronuclei in the peripheral lymphocytes of boars and sows

Donor	Micronucleus frequency per 1000 binucleated cells				
	Boars	Pre-parturient sows	Post-parturient sows	Different reproduction periods sows	Irrespective of sex pigs
1	12.2	4.5	5.3	4.5	4.5
2	5.4	4.2	4.1	4.2	4.2
3	7.9	3.1	9.4	3.1	3.1
4	11.3	4.1	4.1	4.1	4.1
5	10.3	14.2	8.0	14.2	14.2
6	8.7	11.8	10.0	11.8	11.8
				5.3	5.3
				4.1	4.1
				9.4	9.4
				4.1	4.1
				8.0	8.0
				10.0	10.0
					12.2
					5.4
					7.9
					11.3
					10.3
					8.7
Mean	9.3	6.98	6.82	6.9	7.7
Std Dev	2.49	4.75	2.66	3.7	3.45
SEM	1.02	1.94	1.08	1.06	0.81

no significant difference

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