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ANIMAL HUSBANDRY

Karyotypic Instability of Reindeer in Zones of Potential Environmental Problems

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Abstract—The problem of livestock breeding in areas adjacent to industrial centers, which form ecological ill-being, is aggravated year by year. The aim of this article is to determine the degree of genome destabilization in deer—recognized as a source of anthropogenic pollution of the environment—living at different distances from the city of Norilsk. Using methods for determining the frequency of micronuclei and the state of interphase nucleous organizers, a higher destabilization of the deer genome has been found in areas up to 150 km compared to the area of more than 450 km away from Norilsk.

Keywords: reindeer, industrial pollution, micronuclear test, nucleoli, genome destabilization

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INTRODUCTION

Norilsk, located in the northern part of Krasnoyarsk krai, for a long time has been the most polluted city in Russia [1]. Environmental control systems (ecological monitoring, control, audit, etc.) are not enacted by legislation in what concerns company's zone of environmental impact [2]. It has been suggested to define it as a territory, where industrial factors of qualitative and quantitative changes in the environment are registered [3]. In protection of the environment, as well as of soil and plant covers, it is commonly thought that anthropogenic pollution cannot be totally prevented. This testifies to the necessity of studying the state of animal world near different sources of industrial pollution. Genetic screening is used mainly for determining the degree of influence exercised by disturbing environmental factors on the genome of animals. The micronucleus test is performed at the initial stage of monitoring in the majority of countries [4]. The formation of chromosome aberrations is accompanied, as a rule, by abnormalities in interphase nuclei. The frequency of these nuclear abnormalities in micronuclei, caudate nuclei, and chromosome bridges has a high positive correlation with that of individual types of chromosomal disorders, which occur under the effect of radiation, as well as chemical and physical factors [5]. Determining the frequency of micronuclei and nuclear abnormalities is a commonly accepted international test to control the ecological state of soil [6], water basins [7], and even populations of tumor cells [8]. The aim of this work was to reveal the degree of destabilization in the deer genome in areas up to 150 km and more than 450 km away from Norilsk.

MATERIALS AND METHODS

The studies were performed on 44 wild reindeer and domestic reindeer of the Evenk and Nentsi breeds inhabiting areas up to 50, 150, 450, and more km from Norilsk in a direction form north to south. The peripheral blood of deer was taken from the jugular vein. A drop of this blood was placed on a glass plate, air dried, and fixed in 96% ethanol. Then, Romanowsky-Giemsa staining was performed. To determine the frequency of micronuclei in erythrocytes, at least 100000 cells taken from each individual were analyzed. Only morphologically normal, undamaged, cells were studied. The number of genetically aberrant cells was expressed in parts per million. Preparations were examined using an Olympus Vanox-T microscope (Japan). Interphase nucleous organizers (iNORs) were detected by the standard method [9]. Preparations were treated with a 50% solution of colloidal silver nitrate. Simultaneously, two parameters were analyzed in lymphocytes of the examined blood: the number of interphase nucleoli and interphase fibrillar centers. The index of iNORs was calculated as the number of nucleoli in cells divided by the total number of analyzed cells. Statistical processing was performed using Student's t-test.

Table 1. Frequency of erythrocyte and lymphocyte micronuclei in the blood of reindeer

Distance from Norilsk, km	*n*	Erythrocytes		Lymphocytes	
		ppm	CV, %	ppm	CV, %
>450	17	0.011 ± 0.0020*	66.3	0.95 ± 0.180*	35.7
150	13	0.018 ± 0.0015*	34.5	1.92 ± 0.150*	21.4
50	14	0.022 ± 0.0030*	46.2	2.38 ± 0.205*	18.3

* $P < 0.001$ between all parameters.

Table 2. Number of nucleoli and fibrillar centers per lymphocyte

Distance from Norilsk, km	*n*	Nucleoli of lymphocytes		Fibrillar centers	
		number per cell	CV, %	number per cell	CV, %
>450	17	2.38 ± 0.086	12.7	4.65 ± 0.103	17.8
150	13	2.12 ± 0.180	18.6	4.62 ± 0.190	18.3
50	14	2.52 ± 0.213	25.6	5.37 ± 0.147	9.5

RESULTS AND DISCUSSION

The frequency of micronuclei in deer living at a distance of 450 and more km form Norilsk was approximately 0.011 ppm and, thus, was different in a high statistically significant manner from that in deer living closer than 150 and 50 km from Norilsk (Table 1). These differences provide evidence that the genome destabilization worsened near the industrial zone. This is also testified by changes in the frequency of micronuclei in deer lymphocytes. The number of nucleoli per cell did not have any statistically significant differences (Table 2). Nevertheless, the number of fibrillar centers was significantly higher ($P < 0.01$) than in deer living at a distance of 50 km from Norilsk. Differences between the variation coefficients of nucleoli and fibrillar centers number per lymphocyte serve as an indirect proof that the protein synthesis is subjected to anthropogenic influence.

It should be noticed that the blood of animals living at a distance of 50 km contained cells that are not common during the regular hemopoiesis. They were megakaryocytes and blasts, which are typically found in the bone marrow of mammals.

Nonferrous smelters in Russia and foreign countries are regarded as one of the most ecologically destructive industries. Estimation and ecological regulation of the effect produced by these smelters on soils of adjacent areas is a complex research and practical problem. Its solution depends on the choice of most informative parameters of the state of soils and environment. Thus far, the system of ecological regulation has been based on relatively well developed, though outdated, sanitation and hygiene principles and standards [10], which do not comply with the results of genetic screening of animals. According to these standards, the state of soils and natural environment (NE) in the studied area at a distance of closer than 4 km corresponds to the fifth maximal level of ecological quality loss. The area at a distance of 4–16 km may be assigned to the fourth level, and that at a distance of 16–25 km to the third level of ecological quality loss by soils and natural environment. Outside the area of 25 km, the ecological quality of NE corresponds to the first or second levels of ecological quality loss. According to this method, the ecology of an area at a distance more than 25 km from the source of pollution may be considered as relatively favorable. Nevertheless, data obtained in this work indicate that the genotoxic pollution is observed in areas up to 150 km. This is favored by the continuous pollution and prevailing wind direction. Unfortunately, the sanitation and hygiene approach does not take into account ecological features of the environment in the studied area, its resistance to anthropogenic influence, wind rose, and economic peculiarities. Probably, permanent migration of animals plays its certain role in manifestation of the geotoxic influence produced by the environment. Under the conditions of the Extreme North, flora regeneration occurs at a very slowly pace. Thus, lichens, which are the main food objects of reindeer, may turn out to be sources of anthropogenic agents over a long period of time.

Therefore, the obtained results indicate possible long-term consequences of the unfavorable impact on the genome and proteome of deer inhabiting industrially polluted areas adjacent to Norilsk, which should be taken into account when breeding reindeer.

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