
INVESTIGATING THE INFLUENCE OF MILITARY ACTIONS ON ANIMAL GENOM

Slavica Košarčić, Dušan Košarčić, Ranko Kljajić*

*Scientific Veterinary Institute "Novi Sad", Novi Sad, Yugoslavia
*Scientific Institute for Reproduction and Animal Artificial Insemination,
Temerin, Yugoslavia*

ABSTRACT

Contemporary world is still faced with misunderstandings between countries and nations that are solved in militant way. Using war a way of solving the problems, brings universal misfortune and deepens injustice. Applying modern arms destroys environment and leaves harmful consequences on contemporary and future world. Of course, the most dangerous influence is manifested on changes in genetic material of human beings. The aim of the investigation was to discover if there were changes in genome of the animals present in the area where the refinery in Novi Sad was bombed. By the means of random sampling we chose 60 cows and 30 pigs from the area where air, water and food was contaminated with a considerate quantity of harmful matters that could be genotoxic. By sterile procedure according to modified Moorhead's method lymphocytes were cultivated, than the technique of G-banding was used (Seobright et al. 1971) According to the International Standards for Karyotypization of Domestic Animals (ISCNDA 1990) the chromosomes were analyzed. In all the investigation 3 cows with numerical and structural changes type aneuploidia and break on chromatides in q-arm were discovered. Analyzing the genome in all the pigs, 2 animals with structural changes of chromosome type "ring", deletion and break on q-arm (reciprocal translocation) were discovered. According to the percentage of the changes on 100 examined metaphases, it was discovered that the changes in genetic material appeared "de novo" and that there are no constitutional changes in karyotype. Out of 90 animals, 5 with chromosome aberration were discovered, which makes 5.5 %. This shows that the changes in genetic material are a consequence of genotoxic agents.

Key words: military actions, genotoxicity, chromosome, numerical and structural changes

INTRODUCTION

To preserve and protect the environment is an obligation and need of all the inhabitants of our planet. In the process of universal genesis and survival of all the societies and

individuals, it is necessary to realize that protection of the environment cannot be accomplished by divisions, misunderstandings and strict geographical borders that are not and cannot be border for ecology. The most dangerous and most harmful phenomenon for matter circulation in nature and natural eco-systems is using distrustful modern arms and military actions as a way of solving the problems between the states and nations.

From March 24 until June 24 1999 our country, FR Yugoslavia, and their citizens were witnesses of an occasion where military activity was used for solving the conflict. However, it was obvious that even greater injustice was done, many things were destroyed, biological systems were distorted, the environment contaminated with harmful matters that will bring serious consequences on the living beings in the world. During the air raids on FR Yugoslavia, oil refinery in Novi Sad was also bombed. On its equipment and storage 270 missiles with extremely strong destructiv power were thrown. A total of 73.770 tons of oil and its derivatives were destroyed out of which 65.180 tone burned, and the rest was poured in the Danube and its surrounding. In the process of incomplete oil burning a great quantity of harmful matters, poisoning gas and soot were released. According to the Federal Ministry for Scientific Development and the Environment [7], concentration of SO₂ (sulphur dioxide), CO (carbon monoxide) and NO_x (nitrogen monoxide) were high above the permitted values. In the center of the city measured concentration of sulphur dioxide was 380 µg/m³, while tolerant value is 150µg/m³. On our farm the value of the harmful matters were above the permitted level, because the wind was directed from the refinery towards the farm (concentration of SO₂ was 570 µg/m³). The underground water for the cattle was poluted with lead, total carbohydrogen, polycyclic aromatic carbohydrogens and polychlor biphenyls. Besides measurable harmful matters, stress influence on animals could also be noticed. Some cows lost milk, although they were in period of lactation and some pigs were too sensitive and died. These information, together with the results of investigation and changes in the offspring of the animals show that harmful matters that appeared as a consequence of bombing, could not be controlled and were part of life cycle, chain of nourishment and circling the matter in nature. The given matters, according to published investigations, are considered to be genotoxic, because they damage DNA molecules which are the hereditary base for the living beings [2,4].

MATERIAL AND METHODS

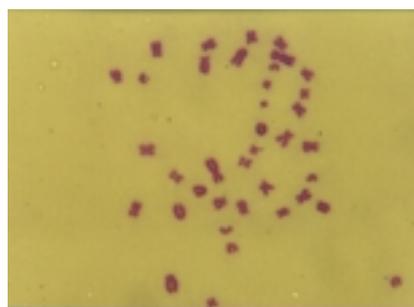
Investigating the influence of harmful matters in animal genome was studied on the area of bombing. On the farm 5 km away from the refinery, where the presence of harmful matters was discovered in air, water and soil, by the method of random sampling 60 cows were chosen. In the same way 30 pigs were chosen in a village near the refinery. According to the information from the Federal Ministry for Development of Science and Environment, concentration of CO₂, SO₂, NO and soot was several times higher that the permitted values. Harmful matters in air, or deposited in underground water and soil, as for example lead, all the carbohydrogens, polycyclic aromatic carbohydrogen and polichloric biphenols were present in extremely high values. Control of these experimental animals was the result of cytogenetic monitoring of breeding material from the previous year done on the same location. From chosen animals by a sterile method, blood was taken from vein jugulars and the culture of lymphocyte was established according to the modified method of Moorhead with a technique of chromosome banding (Seobright et al). The chromosome were analyzed according to the International Standards for Karyotypization of Domestic Animals (ISCNDA 1990) [3].

RESULTS AND DISCUSSION

Chromosome analyses for 90 chosen animals were done by examining 100 metaphases for each animal. For every animal three cultures were established and five preparations made, which made a total of 1350. The facts on cytogenetic control of breeding animals on this farm from 1998 show that in all the animal no changes were discovered. Normal karyotype $2n=60$ XX and $2n=60$ XY, $2n=38$ XX and 38 XY, characteristic for this kind of cattle (*Bos taurus*) and pigs (*Sus scrofa*) respectively, was present in the animals analyzed in the previous year (Picture 1, 2).



Picture 1. Microphotography of normal cattle karyotype $2n=60$ XX



Picture 2. Microphotography of normal swine karyotype $2n=38$ XY

In the chosen group of cows, three cows with numerical and structural changes on chromosomes were discovered. Numerical changes were aneuploidia $2n>60$ and in cow karyotype Tet. No. 38241 two kind of cells were discovered with 11% higher number of chromosome ($2n>60$, $2n=89$ XX) and 23% cells with $2n>60$, $2n=73$ XX (Pictures 3, 4).



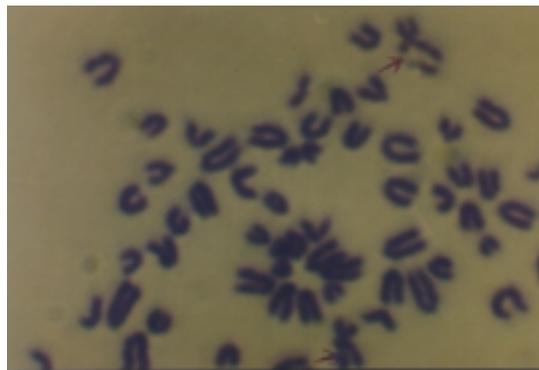
Picture 3. Microphotography of karyotype with aneuplidia $2n>60$, $2n=89$ XX, Gisma stained, 1000 times

These changes appeared as a consequence of chemical and genotoxic matters [6]. Analysing the karyotype of cow Tet. No. 38412 structural change type break on q-arm in one chromatite of both X chromosome, was discovered (Picture 5). Out of 100 examined

metaphases 49% cells had already mentioned structural change. Reproduction disturbances were present in these cases and miscarriage occurred in the first part of gravity.



Picture 4. Microphotography of karyotype with aneuploidia $2n > 60$, $2n = 73XX$, Gizmo stained, 1000 times



Picture 5. Microphotography of karyotype with brake on q-arm of X-chromosome, Gizmastained, 1000 times

From the literature it is known that X-chromosome in *Bos taurus* kind of cows reacts on harmful matters by creating fragile places and breaks on chromatides [4]. In the year 2000 two calves were calved with teratogene changes (2 heads) on the same farm (picture 6). The mothers were not in the experimental group. Since they died during the calving we have no reliable information about the reasons for teratogene changes. However, time of these changes and the changes discovered in the experimental group from this farm are indicative.

In the chosen group of swines we discovered two animals with structural change on chromosome, type of reciprocal translocation and ring chromosome. Translocation that appeared by joining of the broken fragment of q-arm with one chromate of the 14th chromosome and translocation on q-arm of one chromate of the 2nd chromosome was discovered in 36% of the cells out of 100 examined metaphases. It can be stated that changes in karyotype occurred "de novo" as a consequence of harmful matters on the genome [1].



Picture 6. Photography of calves with two heads. Calved in 2000



Picture 7. Microphotography of rcp. (14q+; 2q-) Gizma stained, 1000 times

Since this mutagen change is balanced, genetic material was not lost, no functional changes were discovered, but there were evident disturbances in reproduction and in mortality of embryos. In the literature from our country and abroad reciprocal translocation is considered a serious cause of disturbances in swine reproduction [2]. Discovered "ring" change on chromosome was on one homologous 13 pair and indicates presence of intensive radiation on this area during bombing. This chromosome aberration appeared "de novo" and was discovered in 26% of the examined metaphases.

These changes in the genome of the endangered kind of animals points that the probably there is a genotoxic agents in contaminated environment, yet there is no reliable information on its influence. It is indicative that further investigations are necessary. Introducing reliable methods for controlling the surrounding must be parallel to the investigations done on the culture of cells. From the results we notice that there are no numerical and structural changes in constitutional karyotype (all the cells of organism were included), but the damages occurred "de novo" most probably under the influence of genotoxic matters [1,6].

Available information from literature and those attained through experiments, lead to a conclusion that harmful abiotic and biotic factors in water, air and food damage DNA structure in molecule and thus influence genome on the whole.



Picture 8. Microphotography of "ring" chromosome out of 13th pair, Gizma stained, 1000 times.

CONCLUSION

After completed cytogenetic analyses of 90 animals we may conclude:

The control animals had normal karyotype that is characteristic for the kind $2n=60$ XX and $2n=38$ XY.

In the experimental group that was close to the source of harmful influences, 5 animals (3 cows and 2 swines) were discovered with numerical and structural changes type aneuploidia, brake on chromosome, reciprocal translocation and "ring" chromosome.

Damage on chromosome appeared "de novo".

Harmful matters in air, water and soil on the farm and the village were considerably above the permitted values.

Further investigations should be done and more animals should be included.

LITERATURE

- Bondor Ma, Gooch PC. (1983): Mechanisms of chromozomal aberrations production, Cytogenetics 2107
- Gustafsson A. (1991): Mutation enviroment and evolution, Cold Spring Harbor Symp. Quant 16 263-282
- Di Berardino D., Hayes H., Frifs K., Long S., (1990): ISCND A Cytogenet. Cell. Genet
- Evans H.J. (1982) : Cytogenetic studies on industrial populations exposed to mutagenes, Gold Spring Harbor, New York 325-36
- Llambis, S.- Guevara , K. - Rincon G. -Aruga , M.V.- Postiglioni A. : (1999) Aphidicolin - induced fragile sites in Bos Taurus lymphcrite cultures, Animal production - Hungary vol. 48 1. pp 117-120
- Raport I.A. (1988): Specifichnostt himicheskogo mutageneza, Izd. Nauka, Moskva, 249 pp.
- Federal ministry for development , science and the environment FR Yugoslavia (2000). Report The Consequences of NATO bombing FR Yugoslavia Consequences of NATO bombing FR Yugoslavia