RESEARCH ON SPONTANEOUSLY EMERGED CHROMOSOMAL ABERRATIONS IN THE PERIPHERY BLOOD LYMPHOCYTES IN CATTLE ('BUŠA' BREED)

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Abstract

Knowledge of spontaneous aberrations, namely, of their frequency in non-irradiated cells is of paramount importance not only in cytogenetic research, but also in contemporary animal production.

The paper deals with research on spontaneously emerged chromosomal aberrations in the peripheral blood lymphocytes in the cattle of 'Buša' breed.

To obtain metaphase chromosomes the conventional method of lymphocyte cultivation was used, albeit slightly modified and adapted to the examined animals and the laboratory conditions.

The research findings indicate that a certain percent of spontaneously emerged chromosomal aberrations of chromatid type (gap and break) have been found in the peripheral blood lymphocytes in the cattle of 'Buša' breed.

KEY WORDS: chromosomal aberrations, cattle ("Buša" breed), lymphocytes of peripheral blood, metaphase chromosomes

Introduction

In contemporary animal production genetics, in particular, population genetics plays an important role. Its development is closely linked to the introduction of regular check-ups of production properties of domestic animals. An ever-increasing consumption of food products of animal origin, above all, of meat and milk, and consequently, the issue of cost-effective food production, have impacted the genetic research in the sense of its orientation towards finding more advanced methods for objective assessment of the hereditary factors in production properties of domestic animals. Cytogenetic research enables us to gain a better insight into a chromosome structure. This research is not only significant for its purely scientific merit but also for its application potential, and as such, it provides a backbone in contemporary animal production (1). In our environment there exist a number of physical, chemical and biological agents which have a harmful effect on living organisms. These agents are divided into endogenous agents (deriving from the organism and being a common cause of a spontaneous mutation; in respect of their nature, they can be either genetic or non-genetic) and exogenous genotoxic agents (deriving from natural or artificial sources)(2). In examining chromosomal aberrations at low-dose radiation levels it is crucial to be aware of the nature of emerged aberrations, namely, of their frequency in non-irradiated cells. This is substantiated by the fact that there is no such a thing as a benign radiation dose; even the smallest quantity of radioactive pollution is dangerous and long-term, albeit minimal radiation can have the same effect as the atomic bomb explosion. In the sixties of the 20th century due to the minimal, yet acceptable radiation levels in the nuclear facilities wherein nuclear energy was used for peaceful purposes, the annual death rate of Americans suffering from cancer was 5% higher, but the collateral damage was also manifested in respect of congenital defects, a reduced physical resistance, threats to health etc. (3). Because the consequences of chromosomal aberrations in domestic animals have already been established, including a possibility of their re-emergence in the subsequent generations, many countries have introduced the procedures of cytogenetic analysis. The first cytogenetic studies on cattle date back to 1928, and in that year Kralinger recorded the first chromosomal aberrations (4). A cattle breeding has a prominent role in human diet, food-processing industry and in animal feed processing industry, but also in leather processing industry. In most countries cattle breeding, primarily due to meat and milk production, participates with around 50% in the overall agricultural production (5). It is for this reason that in addition to conducting research on normal cytogenetic properties of cattle, different types of structural and numerical aberrations soon became the subject matter of many scientific studies. Deviations from a standard karyotype have various effects on production properties and the well-being of animals (early death of offspring, congenital malformations in offspring, high abortion rate, and sterility). One of the anomalies (defects) seriously affecting the fertility rate in cattle is Robersonian gene translocation whereby two chromosomes are fused. This anomaly changes the number of chromosomes in cattle (from 2n = 60 to 2n = 59) without provoking a loss of genes, but instead, simply changing their position. After undergoing the above translocation the cattle remain normal in terms of the phenotype, but with reduced reproduction properties. Sick females return to the estrus stage earlier, while zygotes from the germinative cells die early in the embryo stage. The most common type of Robersonian translocation in cattle is t (1;29) – the fusion of chromosome 1 (the biggest chromosome) with chromosome 29 (the smallest chromosome). This translocation is all too common a phenomenon in cattle on the European continent. Chimerism is also an anomaly which affects the reproductive properties of cattle. In females suffering from chimerism there appear XXY sex chromosomes, while in males there appear XX sex chromosomes. Chimeric bulls are commonly sterile, excreting only an abnormal sperm quantity, while female ovaries are rudimentary (6). The cytogenetic researchs on cattle are of great economic importance and benefit. According to the findings of Teksen et al. (7) the reduced fertility, caused by gene translocation, incurs a loss of around $ 250,000 in agriculture. An early detection of chromosomal aberrations enables the interruption of gestation and decreases the time of future gestation which is, in turn, cost-effective. The cytogenetic studies in cattle can be carried out on the peripheral blood, amniotic fluid, fibroblasts, the bone marrow and lymph nodes and as such, they have proven very beneficial in animal production (7). The cultivation of the peripheral blood lymphocytes is the most widespread technique recommended by the IAEA since cytogenetic aberrations are considered to be the most reliable indicator of changes which take place at the cytogenetic level (8). The research on the spontaneously emerged chromosomal aberrations in the lymphocytes in cattle of the domestic breed was the aim of the present pa-

per, whereas basic properties of the karyotype were examined with the purpose of facilitating detection of aberrations and providing a better insight into possible anomalies (such as Robersonian gene translocation, and chimerism) which, otherwise, could not be detected by the standard phenotype procedure.

Materials and Methods

The blood of 40 cattle of the autochtonous breed ‚Buša' was used in our research. The blood from *vene jugularis* was taken out by vein punction and put into sterile heparin coated, vacuum-sealed containers. The lymphocyte cultivation was carried out in compliance with the method described by Moorheadet et al. (9). The sterile flasks were filled with 7 ml of the nutrient culture medium, 2 ml of fetal bovine serum, 0,2 ml phytohaemagglutinin (PHA) and 0,5 ml of the blood. For each animal and each dose the cultivation was carried out in two parallel samples. The lymphocyte cultivation was conducted at 38° Celsius (this temperature corresponds to the body temperature of cattle) and left for 48 hours. After 45 hours of cultivation 0,2 ml of 0,05 % colhicine was added to all samples. In the course of the next three hours all cells, entering the division stage, were locked in the metaphase. Three hours after adding colhicine, namely, 48 hours after the initiation of the lymphocyte cultivation process, all cultures from the flasks were transferred into the centrifugation cuvettes and centrifuged at 1000 g for ten minutes.The next step was separation of the supernatant from the residue. A fresh hypotonic solution of 0,075 moldm^{-3} KCl was added into the residue. The hypotonic treatment lasted for 20 minutes at temperature of 38° Celsius. Thanks to the hypotonic solution the cell volume is enlarged while chromosomes are better placed (positioned). The heating of the hypotonic solution at temperature of 38° Celsius increases efficiency, facilitating a faster transmission of water through the cell membrane and softening the cytoplasmatic membrane, which, in turn, increases the extension capacity of the latter. Following the hypotonic treatment the suspension was centrifuged again, and then the prepared fixative was added to the whitish residue. The fixative is a blend of methyl alcohol and glacial acetic acid in the ratio of 3:1, prepared an hour earlier and cooled at the temperature of + 4° Celsius. During fixation procedure the surplus water was removed from the cells, resulting in their fixation. The cool fixative increases the visibility of chromosome contours. By multiple, consecutive rinsing in the fixative, in addition to centrifuging (at 1000 g for ten minutes) the white residue was obtained (ie. the cell suspension). 0,5 ml of fixative was added to the white residue. The fixative was being stirred and dropped by pipette from a certain height onto the obliquely positioned slides, previously cooled at the temperature of – 20° Celsius. After having been dried at room temperature, the preparations were stained with a 5 % Giemsa solution in the phosphate buffer (Gurr) for 10 minutes. After the staining procedure, the preparations were rinsed in liquid first, and thereafter, in distilled water. Finally, they were dried and numerically labelled. The microscopic cytogenetic analysis of preparations was performed using a BX 41 light microscope. Using an inverted microscope (x1000) all the detected changes were photographed. Analysis of chromosomal aberrations was performed in 48-hour- culture medium. For each animal 100 clearly visible metaphase figures were analysed. The detected aberrations were classified in compliance with the International System of Cytogenetic Nomenclature (ISCN) (10). The metaphase chromosomes in the karyotype are ordered using Adobe Photoshop 6.0. programme, homologue pairs aligned into the karyogram in accordance with their size.

Results and Discussion

The basic properties of the normal karyotype of the 'Buša' breed were represented in Figure 1. The findings of our research indicate that the diploid number of chromosomes in this species is 2n = 60, XX in females, and 2n = 60,XY in males, respectively. The diploid chromosome set consisted of 29 pairs of acrocentric autosomes and one pair of metacentric (submetracentric) sex chromosomes. X sex chromosome was significantly larger in comparison with Y chromosome. The findings in our research, conducted on 40 animals, indicated that there was no deviation from the normal karyotype, which, in turn, corresponded to the results of similar studies (11). Results of the analysis of spontaneously emerged chromosomal aberrations in the peripheral blood lymphocytes of the 'Buša' cattle were presented in Table 1. The presence of spontaneously emerged chromosomal aberrations in the peripheral blood lymphocytes of the examined 'Buša' cattle was asserted (confirmed). We observed the chromatid aberrations: gap (Fig. 2.) and break (Fig 3.), chromosomal aberrations: acentric fragment (Fig. 4.) and dicentric chromosome (Fig. 5.), but in respect of numeric aberrations we recorded polyploidy (Fig. 6.). The presence of various chromosomal aberrations in 'Buša' cattle was evidenced by the analysis of 4000

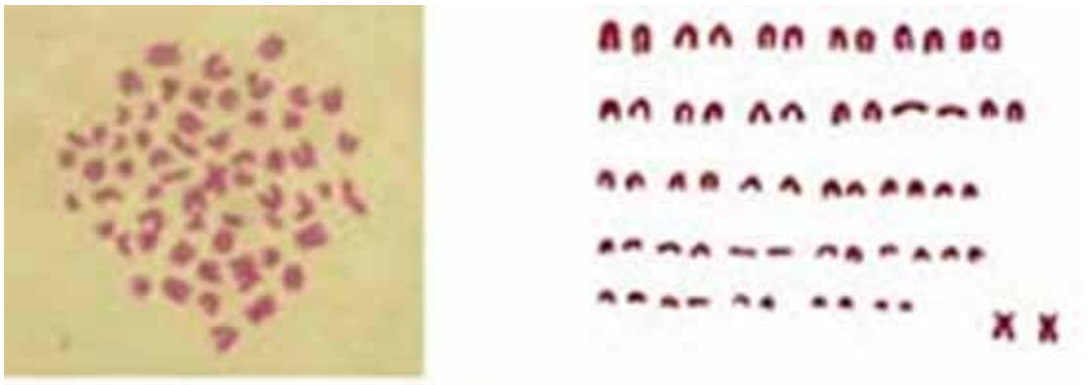

FIGURE 1. Metaphase figure and karyogram of a female 'Buša' cow

Number of research animals	Number of metaphase figures	Chromatid aberrations		Chromosomal aberrations		Numerical aberrations	%
		Gap	Break	Acentric fragments	Dicentrics		
40	4000	15	12	7	4	16	1,35

TABLE 1. Chromosomal aberrations in the lymphocytes of 'Buša' cattle (collective presentation)

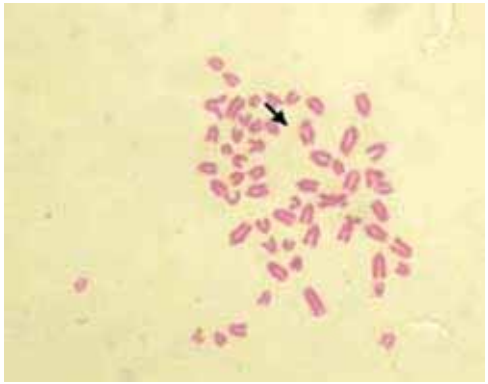

FIGURE 2. Gap

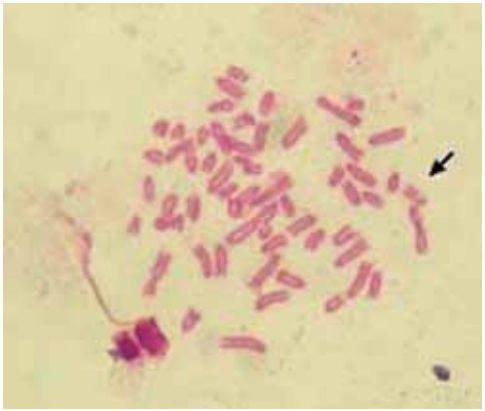

FIGURE 3. Break

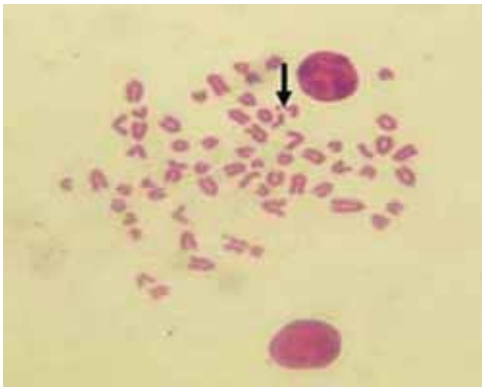

FIGURE 4. Acentric fragment

metaphase figures. The total number of gaps amounted to 15, of chromatid breaks to 12, acentric fragments to 7, and dicentric chromosomes to 4, while the number of polyploidy mitoses amounted to 16. The total percent of spontaneously emerged chromosomal aberrations amounted to 1,35 (Table 1). Our analysis, which was conducted on clearly visible metaphase figures obtained from the peripheral blood lymphocytes of 'Buša' cattle, has confirmed the findings of a great number of authors regarding the presence of a certain percent of spontaneously emerged chromosomal aberrations (12,13,14). The percent of spontaneously emerged chromosomal aberrations in our research was 1,35. According to Slijepčević (15) the

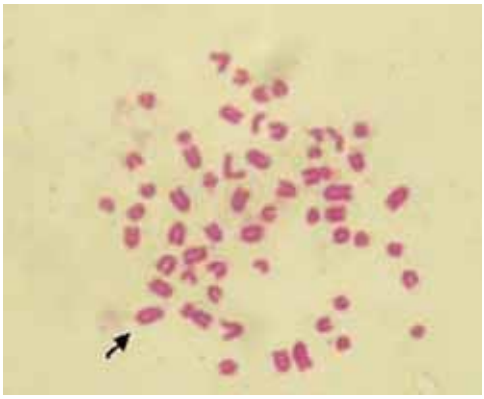

FIGURE 5. Dicentric chromosome

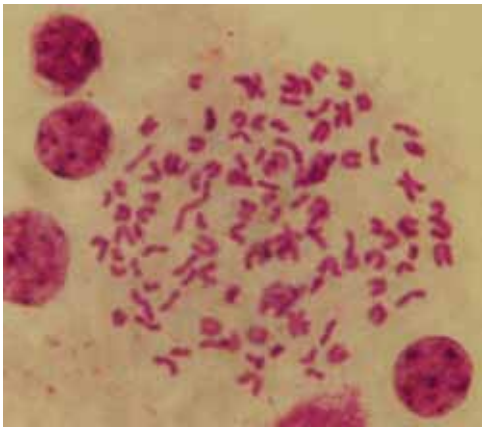

FIGURE 6. Polyploidy mitosis

percent of spontaneously emerged chromosomal aberrations in the lymphocytes of swine was 0,77, in goats 1,40 (1), in horses 1,08 (16), while in cattle this percent was 1,62 (4). As far as people are concerned, data vary significantly while the most comprehensive data on the level of spontaneous aberrations were recorded by Zaharov (17). On the population sample of 60 000 people of either sex, ranging from 0 to 70 years in age, he found the frequency of spontaneous aberrations to be 1,02%. The underlying reasons for inter-species differences in the biological answer to the percent of spontaneous aberrations should be searched for within the context of specific features inherent to a particular species. There are numerous factors which can influence a different biological answer of an organism to the emergence of chromosomal aberrations while repair mechanisms, age status and the overall condition of an animal are but some factors which must be taken into account in recognizing and assessing this problem (18). In the samples of the examined 'Buša' breed cattle the chromatid aberrations of the types gap and break were most common. The chromatid type of aberrations manifested as spontaneous aberrations is almost invariably present in healthy individual specimens. Chromatid breaks represent at the same time the genuine chromatid lesions. They appear spontaneously in 1 do 2 % of normal metaphases. The most commonly observed chromatid aberration is a gap or „chromatid thinning", ie. the unstained achromatic chromatid region. Gap cannot be classified as a genuine damage since it does not obstruct the continuity of the chromosome structure and it appears a normal phenomenon in as much as 2 do 4 % of the analysed metaphases of healthy cells. These changes are induced following the adding of a mutagene stimulant and they provide only a technical background, while the chromosomal type of aberrations clearly indicates to a relationship of cause-effect (4). The recorded polyploidy mitosis (polyploidy) is a result of nonefficiency of the division spindle. In analysing the types of chromosomal aberrations in 'Buša' cattle it was noted that the acrocentric morphology of chromosomes renders their identification more difficult, especially in the so called instable aberrations (dicentrics). As a rule, aberrations disturb the gene balance and can influence not only fertility, but also the productivity of domestic animals. Chromosomal aberrations cause significant losses in potential productivity due to structural and numerical changes which take place in the gene type. An increasing use of nuclear energy for medical, industrial and other purposes makes it an imperative to monitor closely the genotoxic damage both in humans and animals, but also a need for further exploration of the territory and nature of aberrations. A further research of spontaneous aberrations will be focused on other cattle breeds on the territory of Bosnia and Herzegovina in order to compare potential differences with the percent of aberrations found in the autochtonous cattle breed of 'Buša'.

Conclusion

On the basis of results obtained in the research of spontaneously emerged chromosomal damage in 'Buša' cattle by applying the cytogenetic method of testing in the lymphocyte culture, the following general conlusions can be drawn:

- The diploid number of 'Buša' chromosomes is 2n = 60,XX in females, and 2n = 60,XY in males, while the chromosome set consists of 29 pairs of acrocentric chromosomes. Sex chromosomes are metacentric (submetacentric), while the X sex chromosome is significantly larger than Y chromosome.
- A certain percent of spontaneously emerged chromosomal aberrations was noted in the peripheral blood lymphocytes of 'Buša' cattle.
- Spontaneously emerged chromosomal aberrations in the peripheral blood lymphocytes of 'Buša' cattle were most commonly of the chromatid type – gap and break.
- Cultivation method of the peripheral blood lymphocytes proved to be very suitable for obtaining metaphase chromosomes which, in turn, enabled an easy detection of potential changes at the cytogenetic level.

List of abbreviations:

IAEA - International Atomic Energy Agency
ISCN - International System of Cytogenetic Nomenclature
PHA - phytohaemagglutinin

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