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# KARYOLOGICAL DATA ABOUT THE BULGARIAN NATIVE DOG BREED “KARAKACHAN DOG”

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## ABSTRACT

*The Karakachan is a mountain sheep dog belonging to a rare livestock protection breed. The Karakachan dog is a breed originated in Bulgaria and is a descendant of the dogs bred by the Thracian nomadic shepherds.*

*The aim of the present investigation is to approbate and optimize an appropriate methodology to investigate the dog's chromosome set without disturbing the animals vital functioning. For this purpose bone marrow cells dragged out of the sternum of male and female individuals aging between 3.5 to 15 years were used. The yield material was cultivated 24 hours at 37°C in RPMI medium with 10% of FBS and 2 mM Lglutamine.*

*Metaphase plates for karyological analysis were obtained by preliminary treatment of the express cell culture with colchicine (0.4 mg/ml culture medium), incubated with 0.075 M potassium chloride at 37°C, fixed in cold 1:3 glacial acetic acid: methanol and stained in 5 % Giemsa solution (Sigma Diagnostic).*

*The chromosome set of the karakachan dog consists of 78 chromosomes, 38 pairs of which are acrocentric autosomes and one pair of sex chromosomes. In some of metaphase plates structural chromosome aberrations were observed – breaks and fragments. Polyploid cells were also counted. These chromosome damages could be due either to endogenous factors connected to the animals' age or to the exogenous influence of the environmental genotoxicants. The optimized protocol could be easily applied to any other dog breed and in wolfs for karyological studies.*

**Keywords:** chromosome, chromosome aberrations, dog karyotype, karakachan dog

## Introduction

The native Karakachan breeds of horse, sheep, and dog are among the oldest of domestic animals in Europe, providing a genetic bridge between modern high-productive breeds with their ancestral, undomesticated ancestors. To its credit, the Karakachan dog has been both historically and traditionally the only effective and most suitable protection against flock predators.

The Karakachan Dog is one of Europe's oldest dog breeds (3). A typical Mollos, created for guarding its owner's flock and property, it does not hesitate to fight wolves or bears to defend its owner and his family in case of danger. Its ancestors started forming as early as the third millennium BC. The Karakachan Dog is a descendant of the dogs of the Thracians – the oldest inhabitants of the Balkan peninsula, renowned as stock-breeders, whom Herodotus describes as

the most numerous people after the Indian one. The Proto-Bulgarians also played an essential part in the formation of the Karakachan Dog as they brought their dogs with them at the time of their migration from Pamir and Hindukush.

The dog is named after the Karakachanians - nomadic shepherds of Thracian origin. Due to their conservative stock-breeding traditions, they managed to preserve some of the oldest breeds of domestic animals in Europe – the Karakachan sheep, the Karakachan horse, and the Karakachan Dog. It is with this name that the Karakachan Dog appears in the works of some of the classics of Bulgarian literature, namely Yordan Yovkov, Georgi Raitchev and Yordan Radichkov. In 1938 H.B. Peters wrote about it in the German cinological magazine. The first researcher of the breed was Todor Gaytandjiev, who proposed the standardization of the breed in the 1940s.

Nowadays these dogs are found in the Bulgarian mountains - Rila, Pirin, Rhodopes, Stara Planina, where they are used as herding dogs to escort flocks of livestock in

Bulgaria. Descendants of Karakachan dogs are also found in northern Greece and Macedonia, through which territories the Karakachans had passed.

Standardization of the Karakachan dog breed was proposed in the 1970s. During recent years, cytogenetic studies of dog chromosomes have focused on establishing the standard karyotype and physical localization of the marker loci (2). Karyotyping of dog chromosome set is a difficult task due to the high diploid number ( $2n = 78$ ) and the similar morphology of autosomes, all of which are acrocentrics.

The aim of this investigation is to describe the karyotype of the Karakachan dog. There are no data found concerning the Karakachan dog karyology which makes this investigation certainly actual. In order to be successful these karyological studies, approbation and optimization of the protocol for metaphase chromosomes suitable for analysis is required. This approach provides preservation of the animals vital functioning.

## Materials and methods

In this investigation 5 male and 3 female specimens aging between 3,3 to 15 years were used. The Karakachan dogs have grown in the vicinities of Sofia, village of Manolsko konare, Plovdiv region, Pleven, Haskovo, Karlovo.

Bone marrow cells were collected from the animals' sternum under anesthesia. The material was transported to the laboratory in ice box at 4-7°C in RPMI medium with 10%

fetal bovine serum, 2 mM Lglutamine and Gentamicine.

The yield cell material was washed twice in fresh RPMI and cell suspensions were obtained by pipetting of the small pieces and cultivated in tissue culture dish for 24 hours at 37°C in the culture medium.

Metaphases for karyological analysis were obtained by preliminary treating of thus prepared express cell cultures with colchicine (0.4 mg/ml culture medium). It was found that the 45 min period is the most appropriate duration of the colchicine treatment procedure. The yield cell material was incubated with 0.075 M potassium chloride at 37°C, centrifuged and fixed in cold 1:3 glacial acetic acid:methanol using a method applied by our work team for small mammal chromosome slides (1). After fourfold fixation the obtained cell suspension was dropped on wet cold microscope slides. Air dried slides were stained with 5 % Giemsa solution (Sigma Diagnostic).

Karyological analysis was carried out using light microscope Zetopan, Reichert x100 oil immersion objective.

This modification represents a version of widely used in various laboratories approach to obtain chromosome slides from cultivated cells in vitro.

## Results and Discussion

The results of the karyological analysis are presented on **Table 1**. Metaphases with well outlined chromosome morphology were counted.

**TABLE 1**

Chromosome aberrations and polyploidy in 8 specimens of Karakachan dogs.

\*The number of the metaphases analyzed depends on the cell culture mitotic activity.

Name and age	Number of metaphases scored*	Type of aberrations					Polyploid cells	Percentage of cells with aberrations
		Breaks	Fragments	Exchanges				
				c/c	t/t	c/t		X ± % EΣ
Valia - 3.3 years	31	1	1	0	0	0	2 (6.45%)	6.45
Sirak 2 - 3.9 years	42	2	1	0	0	0	2 (4.76%)	7.14
Murtap - 11 years	25	2	1	0	0	0	3 (12%)	12
Rim - 15 years	6	0	0	0	0	0	1	0
Paijo 2 - 9 years	32	2	0	0	0	0	1 (3.13%)	6.25
Fatma - 7 years	21	2	0	0	0	0	2 (9.52%)	9.5
Sara - 9 years	17	1	0	0	0	0	1 (5.89%)	5.9
Harry - 3.4 years	4	0	0	0	0	0	2 (6.45%)	9.67
							6.89 ± 1.12	8.13 ± 0.87

The investigated cell populations were characterized with extremely low proliferative activity – less than 1%, which

requires preparation and analysis of larger number of slides when compared to similar investigations concerning various species small mammals. The analysis showed that the bone marrow cells obtained from younger animals of all 8 dogs (Valia and Sirak 2) possessed satisfying proliferative activity. For some of the animals, Rim for example, only a single mitosis in all analyzed chromosome slides was found.

All investigated male and female Karakachan dog individuals have chromosome number  $2n=78$ . At the basic cytogenetic level, the karakachan dog karyotype consists of 38 pairs of acrocentric autosomes gradually diminishing in size. These features make identification of homologous pairs by conventional analysis rather difficult. Sex chromosome pair is represented by two large metacentric X in females. The Y chromosome in males has metacentric morphology and is the shortest one in the complement. Therefore a total number of chromosome arms (NF) is 80 (Fig. 1). Deviations concerning the chromosome number in the sex chromosome pair (XXY, XO, OY for example) were not observed. In all analyzed male and female animals a normal configuration of the sex determining chromosome pair was observed – XX for females and XY for male (Fig. 1)

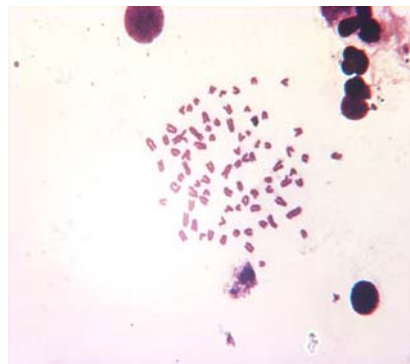


Fig. 1. Karyotype of male Karakachan dog (Sirak 2)

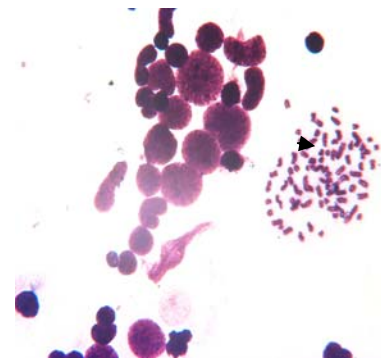


Fig. 2. Metaphase with chromatid break

In some of metaphase plates structural chromosome aberrations (breaks and fragments) were observed (Fig. 2, 3). The percentage of metaphases with aberrations was  $8.13 \pm$

0.87. Polyploid cells were also counted (Fig. 4). They are  $6.89 \pm 1.12\%$ .

These findings support the data about numerous chromosomal abnormalities which have been described, including centric fusions, sex chromosome aneuploidy and polyploidy in normal (6) and in canine tumour tissues (7).

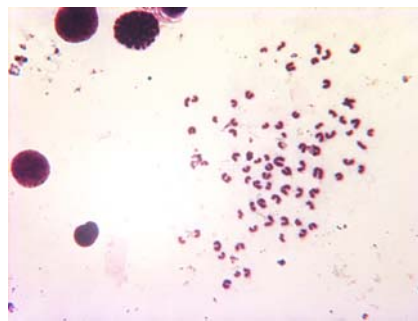


Fig. 3. Metaphase with fragments

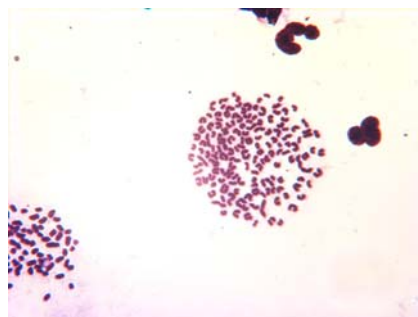


Fig. 4. Polyploid cell in bone marrow of Karakachan dog. Sex chromosomes are also visible.

Because of the large chromosome number the canine karyotype is difficult for investigation and precise identification of particular chromosomes. Namely these special features as well as the extraordinary interest towards this species and the presence of a great number of breeds (the dog is represented by more than 700 breeds) have imposed a karyotype standardization using various banding techniques (10). Besides this there is no conventional standard for dog chromosomes karyotyping (arranging of chromosome pairs). According to Reimann et al. (9) depending on the type of study and the resolution of bands on the chromosomes, basically two nomenclatures should be used in the future. One of these nomenclatures was described by the standard Committee (10), and the other by Reimann et al. (9).

Strict recommendations concerning the use of a nomenclature for the description of chromosomal aberrations in the canine karyotype have not yet been made. We have abided by the recommendations of ISCN (5).

## Conclusions

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In conclusion:

- this optimized protocol could be easily applied to any other dog breed and in wolfs for karyological studies.
- the number and morphology of bone marrow cell chromosomes of the Karakachan dog breed does not differ from the domestic dog (*Canis familiaris*, L. 1758) standard karyotype which was reliably described by Hare et al. (4).
- in the Karakachan dog karyotype were observed relatively high percentage of cells with structural and numerical chromosome aberrations.

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