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Research Article

Analysis of the Cytogenetic Status, Genome Instability, and Adaptive Potential of the Kazakh Tobet Dog

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Abstract: In recent years, interest in indigenous dog breeds has increased. The Tobet is a unique breed of Kazakh livestock guardian dogs adapted to nomadic pastoralism that developed over thousands of years across Central Asia and Turkestan. Currently, the Tobet breed faces critical endangerment. Its conservation and sustainable breeding require thorough analysis of hereditary characteristics, genome stability, and adaptive potential. In this study, 43 Tobet dogs were examined for karyotype, genomic instability, and adaptive potential through chromosomal aberration analysis. Comparative analysis included outbred dogs (n = 15) and Central Asian Shepherd Dogs (n = 15). Novel chromosomal features and heteromorphisms were identified in Tobet karyotypes that were absent in comparison groups and unreported in the literature. Tobets demonstrated significantly greater genomic instability compared to outbred dogs. The elevated genome instability, Robertsonian translocations, and sex chromosome leukocyte chimerism observed in some individuals raise concerns regarding their suitability for breeding programs. Data indicate enhanced adaptive potential and lower genome sensitivity to extreme temperatures in Tobets compared to outbred dogs, consistent with their evolutionary history in harsh continental climates. Early-stage cytogenetic analysis enables rapid assessment of breeding value and health status, emphasizing the importance of continuous genetic monitoring for effective conservation and selection strategies. These findings provide a foundation for evidence-based breed conservation programs and inform breeding decisions to maintain genetic diversity while minimizing deleterious chromosomal abnormalities.

Keywords: Tobet Dog, Canine Cytogenetics, Genomic Instability, Karyotype Analysis, Chromosomal Aberrations, Breed Conservation, Indigenous Dog Breeds, Central Asia

Introduction

Kazakh Tobet dogs have been the companions and protectors of the Kazakh people for centuries. This breed is not only a unique genetic resource, but also an ancient national, cultural and historical heritage of Kazakhstan. For generations, Tobet dogs have guarded the nomads' herds, protected them from wolves and even served as working animals. Unfortunately, this unique breed is almost extinct. There are very few purebred Tobets left. There is a considerable risk that the breed will be threatened with extinction in the near future. The reasons for this are the reduction in grazing areas, the decline in sheep farming, the displacement of the breed by other livestock guarding dogs, uncontrolled breeding, genetic assimilation with other breeds and, above all, the lack of a scientifically based breeding program. The current situation calls for urgent measures to preserve the Tobet breed.

Recently, efforts to preserve, develop and restore the national dog breeds of Kazakhstan (Perfilyeva et al., 2024), including the Tobet, have been supported by the government. The success of these initiatives depends

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largely on systematic breeding programs, which must be based on scientific principles. The modern arsenal of selection methods has expanded considerably thanks to advances in genetics, genomics, cytogenetics and biotechnology. Their integration into breeding programs has ensured steady progress in the management of both productive and unproductive animal breeds worldwide. However, none of these methods have yet been applied to the Tobet breed in Kazakhstan.

These approaches are particularly important for breeds that are threatened with extinction. In such cases, genetic research is the only way to obtain crucial information on the parameters of genetic diversity and the adaptive potential of the remaining representatives, which will enable the development of an effective strategy to restore the breed.

One of the genetic tools that can provide valuable information to breeders and veterinarians working with the breed is the assessment of genetic status, genome stability and indicators of adaptive genetic potential (Reimann-Berg et al., 2012).

The evaluation of karyotype and genome instability within a breed provides breeders with important information to exclude undesirable genetic defects in the population and to favor individuals with more stable genomes for breeding. In addition, an increase in the frequency of chromosomal aberrations, as one of the manifestations of genome instability, reflects a higher rate of structural changes in DNA, such as mutations. On the one hand, this is a characteristic feature of various diseases, including cancer. On the other hand, genome instability itself increases the risk of their occurrence and plays an important role in the progression and dissemination of pathological conditions. Unlike humans, the genomes of dogs, especially indigenous breeds, are less well studied in terms of chromosomal abnormalities (Chatterjee et al., 2017; Udroiu and Sgura, 2017).

Various chromosomal morphological abnormalities have been widely described in productive domestic animals (Dzitsiuk and Tipilo, 2017; Holečková et al., 2021; Raudsepp and Chowdhary, 2016; Šutiaková et al., 2013). However, cytogenetic studies in dogs are extremely limited and mainly focus on numerical karyotype abnormalities associated with tumors and reproductive disorders (Dickinson et al., 2016; Morais et al., 2017; Sargan et al., 2005; Szczerbal et al., 2021; Thomas et al., 2003). Given the lack of such studies, an evaluation of genome instability and adaptive potential in Tobet dogs compared to outbred dogs (free-ranging dogs/outbred dogs dogs) was performed. These dogs serve as a model for genetic diversity due to their high adaptive potential and are hypothetically the most genetically adapted representatives of *Canis familiaris* to environmental changes (Pedersen et al., 2013).

The aim of assessing adaptive potential is to identify genetic traits that ensure the survival and adaptability of a population to changing environmental conditions with increased exposure to stress factors of various origins, such as radiation, chemical exposure and other anthropogenic factors (Pirnajmedin et al., 2024). Adaptive responses are primarily achieved through increased functional activity of cells, organs and systems. Under optimal living conditions, adaptive responses are minimized, and energy is provided for ongoing vital processes. When stress factors exceed the optimal limits, the organism begins to activate adaptive mechanisms. Assessing the adaptive potential of a breed helps breeders to select and breed animals that are better adapted to adverse conditions to improve the breed's resilience (Ilardo and Rasmus, 2018; Rocha et al., 2021). The aim of this study was to assess the cytogenetic status, genome instability, and adaptive potential of Tobet dogs by analyzing chromosomal abnormalities in peripheral blood lymphocytes, in order to evaluate their prospects and suitability for future breeding programs.

Materials and Methods

Study Subjects

The study involved 43 Tobet dogs, 15 Central Asian Shepherd Dogs, and 15 outbred dogs of different ages and sexes. Outbred dogs were used as the control group to represent genetic diversity. The Central Asian Shepherd Dog was included in the study as a comparison group because Tobets share similarities with this breed in terms of phenotype, geographic distribution, and their use as livestock guardian dogs. Peripheral blood samples were collected from all subjects. To assess the cytogenetic status and genome instability, the frequency of Chromosomal Aberrations (CA) in the lymphocytes was analyzed.

To analyze possible sex- and age-related differences in cytogenetic data, Tobet dogs were divided by sex (20 males, 23 females) and into three age groups according to their biological age and show classification: 1- 0-9 months (puppy class) (average age: 6 months, 10 individuals); 2- 10-15 months (junior class) (average age: 12 months, 10 individuals); 3-1.5–8 years (adult dogs) (average age: 3 years, 23 individuals).

To evaluate adaptive potential, blood samples from 15 Tobet dogs and 15 outbred dogs were used. These samples were exposed in vitro to physical factors (elevated temperature; see below).

The biomaterial from the Tobet dogs was collected during special expeditions, dog shows and other cynological events. The assessment of the dogs' conformity to the Kazakh Tobet breed standard was carried out by qualified cynologists of the Republican association of Public Associations of Hunters and Hunting Entities "Kansonar". This assessment was based

on the breed standard for the Kazakh Tobet established by Order No. 101 of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan dated March 30, 2023 " On the Approval of Dog Breed Standards of the Kazakh Breed" (Order No. 101 from March 30, 2023). A typical representative of the Tobet breed is shown in Fig. 1.

The study also included comparison groups consisting of outbred dogs and Central Asian Shepherd dogs. The biomaterial from outbred dogs was collected from animal shelters in Almaty.

At the same time as the biological samples were collected, photographic documentation of the dogs was carried out, accompanied by interviews with their owners. The data collected included information about the owner, the age of the dog, its origin, its place of residence and its physical and morphometric characteristics. Prior to sample collection, all owners gave informed consent for their dogs to participate in the genetic study. Biological sampling was performed once for each dog.

Cultivation and Preparation of Canine Chromosome Slides

The cultivation and analysis of dog chromosome preparations were carried out in accordance with the recommendations Klenovitsky and Grishin, 2010.

Cytogenetic analysis of metaphase chromosomes was performed using a Zeiss Ax-ioImager Z.2 motorized microscope with Metafer-4 software with oil immersion at 10 × 63 magnification. The analysis evaluated the number of aberrant cells and the ratio and types of aberrations per 100 metaphases analyzed. All types of chromosomal abnormalities detectable by routine staining were counted. On average, 196 metaphases (min 100, max 480) were counted per dog of the Tobet breed, per Central Asian Shepherd dog 130 metaphases (min 100, max 150), per outbred dog 163 (min 100, max 240). The international nomenclature of canine chromosomes (Klenovitsky et al., 2013; Switonski, et al., 1996) was used for the karyotype study.

Chromosome analysis was performed on coded samples using the blind method to avoid bias in the results.

Fig. 1: A typical representative of the Tobet breed (male dog)

The identified chromosomal abnormalities were classified as chromosomal aberrations (paired breaks and fragments, centromeric rings, and dicentric chromosomes) and chromatid aberrations (single breaks and fragments, interstitial deletions, and chromatid exchanges). Robertsonian Translocations (RT), quantitative abnormalities of sex chromosomes, and genomic abnormalities (polyploidy, endoreduplication) were also recorded.

Photo documentation of the most characteristic and conspicuous chromosomal abnormalities was performed using digital video cameras.

Assessment of Adaptive Potential in Dogs

The adaptive potential of dogs was evaluated by exposing their blood lymphocytes to thermal factor in vitro, whole blood samples from the dogs were heated for 15 minutes at a temperature of 42°C (critical body temperature for dogs (Parker, 2024)). Further cultivation of lymphocytes, preparation of preparations, and their analysis were performed as described above.

Methodological limitations

Sample size.

The limited sample size of Tobet dogs (43 individuals) is due to several factors:

1. The breed is on the verge of extinction, and their population is physically limited
2. Some owners declined to participate in the study for subjective reasons
3. Because cytogenetic studies require blood samples to be delivered to the laboratory within 24 hours, the sampling was restricted to dogs living in southern and central Kazakhstan

Lack of preliminary research

No cytogenetic studies have previously been conducted on Tobet dogs. Furthermore, no studies assessing the adaptive potential of dogs based on in vitro exposure to stress factors (such as critical temperature) were found in the available literature.

Statistical Analysis

For the statistical calculations, the arithmetic mean and its deviation (M ± SE) were calculated as a percentage per 100 cells. The coefficient of variation was calculated. The significance of differences between means was assessed using Student's t-test, ANOVA (F-test), and effect size (Cohen's d). The threshold for statistical significance was set at $p \leq 0.05$.

Results

Analysis of Tobet Dog Karyotypes

The cytogenetic analysis of the Tobet dogs showed

that all the animals examined, except for one female, had no constitutional chromosomal abnormalities. The analyzed karyotype corresponded to the standard canine karyotype. The diploid chromosome number was 2n = 78. The autosomes consisted of 38 pairs of acrocentric chromosomes, which formed a gradually decreasing sequence, and one pair of sex chromosomes. The X chromosome was submetacentric and the same length as the largest autosome, while the Y chromosome was the smallest metacentric chromosome in the set. Figure 2 shows the normal metaphase distributions and karyograms of a male and female Tobet dog.

Sex chromosome chimerism (78XX/78XY) was found in three female Tobet dogs. The Y chromosome was present in a subgroup of the lymphocytes examined (up to 6%). However, in most cells, the genetic sex determined by the sex chromosome set corresponded to the phenotypic sex.

A particularly remarkable result of this study was the identification of a female with a newly described case of a mosaic karyotype. In the leukocytes of this dog, a high percentage (80%) of a cell clone with an X isochromosome was observed. These cells contained 78 chromosomes, including 77 acrocentric chromosomes and one metacentric (X chromosome). The X isochromosome showed a deletion of either the upper or lower arm (78,XX/78,XXq-). Due to the lack of differential staining, it was not possible to determine with certainty which arm was missing, but it is suspected to be the long arm (Fig. 2).

The lack of marked phenotypic or clinical abnormalities in this female may be explained by several factors: The mosaic nature of her karyotype, the partial rather than complete deletion of the chromosome, or the restriction of the chimeric condition to leukocytes rather than affecting the whole organism. This type of anomaly has not yet been described in dogs. While autosomal aneuploidy has been documented in certain animal species (Klenovitsky et al., 2013), no such cases have yet been reported in dogs.

Some individual variations in chromosomal structure were observed. Heteromorphism was observed in the length of the short arms and the morphology of the telomeric regions of the long arms, which appear as attenuated regions or closely spaced satellite structures on chromosome 1. In one dog, satellite structures were identified in the telomeric regions of the long arms of a pair of chromosomes within pairs 25–30. These structural heteromorphisms have not previously been described in the literature for canine chromosomes. In addition, two females showed heteromorphisms in the length of their X chromosomes (Fig. 3).

Analysis of Genome Instability in Dogs Based on the Frequency of Chromosomal Abnormalities

The results of the study on the frequency of CA in the examined dogs are presented in Table 1.

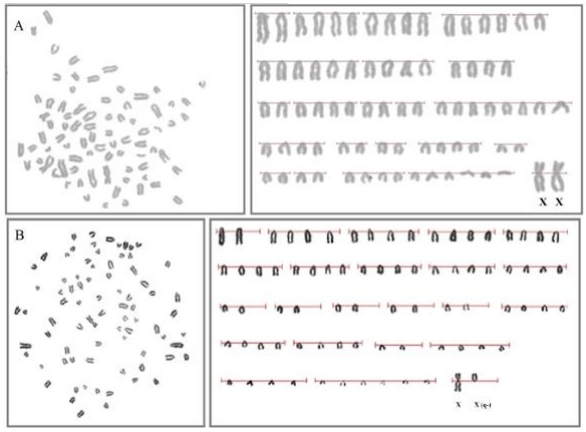

Fig. 2: Metaphase plates and karyograms of Tobet dogs. A - female with normal karyotype (78, XX); B - clone of cells with abnormal karyotype (78,XX/78,XXq-)

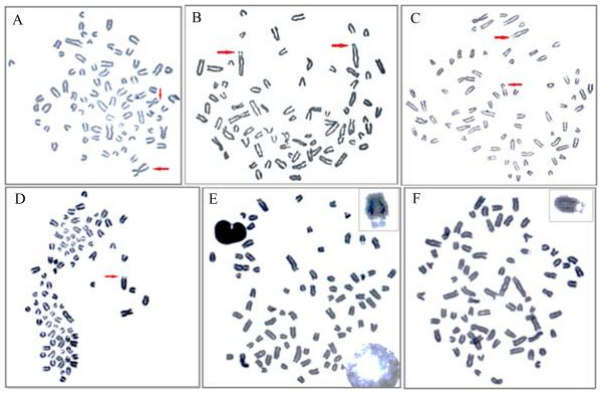

Fig. 3: Heteromorphisms of the chromosomes detected in Tobet dogs. A - heteromorphism of the X chromosomes; B - heteromorphism of the short arm of the first pair of chromosomes; C, D - heteromorphism of the telomeric regions of the first pair of chromosomes and chromosome breakage; E, F - heteromorphism of the telomeric regions of the long arms of one of the chromosomes of the 25-30 pair

Table 1: Spontaneous frequency of chromosomal abnormalities in the studied dogs

Variant	Tobet	Central Asian Shepherd	Outbred dogs
Total cells	8436	1960	2440
Total aberrations	3.08±0.19	3.75±0.61	2.29±0.30
Chromosome type	1.18±0.12	0.42±0.21	0.74±0.17
Chromatid type	1.9±0.15	3,33±0.58	1.56±0.25
RT	0.53±0.08	0.83±0.29	0.95±0.19
Sex chromosome disorders	0.43±0.07	0,21±0,15	0.33±0.12

RT - Robertsonian Translocations $p \leq 0.05$ (t-test, ANOVA) differences are statistically significant between purebred and outbred dogs

Assessment of the spontaneous level of chromosomal abnormalities revealed statistically significant differences in genetic stability between Tobet dogs and outbred dogs when analyzing the total frequency of CA ($p \leq 0.05$, F (1, 56) = 2.31, d = 0.2; t (56) = 2.2) and chromosome-type aberrations ($p \leq 0.05$, F (1, 56) = 2.17, d = 0.2; t (56) = 3.15). The highest genome instability, compared to outbred dogs, was observed in Central Asian Shepherd Dogs in terms of total CA level ($p \leq 0.05$, F (1, 28) = 8.37, d = 0.55; t (28) = 2.15) and chromatid-type aberrations ($p \leq 0.05$, F (1, 28) = 17.17, d = 0.78; t (28) = 2.8).

Chromatid-type aberrations predominated in the spectrum of aberrations, which is characteristic not only of dogs but also of other animals and humans.

In the study of CA in dogs, all observed types of chromosomal abnormalities were recorded. Both numerical and structural chromosomal changes were detected. Numerical abnormalities included single endoduplications and polyploid cells. Structural chromosomal abnormalities included both chromosome type and chromatid type abnormalities.

The spectrum of chromosome type aberrations included paired breaks, translocations and ring chromosomes, while chromatid type aberrations consisted of breaks, interstitial and terminal deletions, exchanges and Robertsonian translocations (RT). Purebred dogs showed an increased tendency for interstitial deletions: 0.33±0.06% ($p \leq 0.05$, F (1, 56) = 11,1, d = 0,45; t (56) = 3,2) in Tobet dogs and 1.04 ± 0.32% ($p \leq 0.05$, F (1, 28) = 25,35, d = 0,95; t (28) = 2,96) in Central Asian Shepherd dogs, compared to 0.08±0.05% in outbred dogs. According to Avet-Louseau et al., the frequency of interstitial deletions in human acrocentric chromosome 13 accounts for 3.7–15% of all deletions of this chromosome (Avet-Louseau et al., 2000; Fonseca et al., 2001). Since all autosomes in the canine karyotype are also acrocentric, we used these values as a reference. Interstitial deletions accounted for 28% of all deletions in Tobet dogs, 55.6% in Central Asian shepherd dogs and 16.3% in outbred dogs. The rate of spontaneous occurrence of RT was significantly higher in outbred dogs compared to Tobet dogs ($p \leq 0.05$, F (1, 56) = 2,82, d.=.0,2; t (56) = 2,03).

Some publications have reported that endogenous factors such as sex and age do not significantly contribute to the increase in genomic damage observed in purebred dogs (Caliri et al., 2023; Santovito et al., 2024). For a comparative assessment of Tobets based on these criteria, an analysis of their cytogenetic data by age and sex was conducted. No significant differences were found among Tobet dogs based on sex. A comparative cytogenetic analysis of the Tobet and outbred dogs by sex (Fig. 4, A) revealed no statistically significant differences in the frequency and type of chromosomal abnormalities in the females. However, these indicators were significantly higher in the Tobet males than in the outbred dogs ($p \leq 0.05$, F (1, 25) = 8,48, d = 0,58; t (25) = 4,79). At the same time, RT were significantly more frequent in both the outbred females and the outbred males ($p \leq 0.05$, F (1, 31) = 6,95, d = 0,47; t (31) = 2,06) than in the Tobet dogs. The frequency of RT was almost six times higher in outbred females than in Tobet females. However, RT were detected in 8% of cells from one male Tobet dog.

Analysis of cytogenetic abnormalities in Tobet dogs of different age groups (Fig. 4B) showed that in young dogs during growth and sexual maturation (groups 1 and 2), the frequency of HA was practically the same. However, their overall HA frequency differs statistically significantly from that of adult dogs ($p \leq 0.05$, t (31) = 3.54). This is probably due to the relative immaturity of the cellular component of the immune system in young animals, which is responsible for the elimination of cells with cytogenetic abnormalities and natural selection, resulting in the survival of dogs with stronger immunity and lower genetic instability (Santos et al., 2023; Cherednichenko et al., 2024).

Isolated cases of polyploid cells and cells with endoreduplication were observed in some dogs. Typically, a small percentage of such cells is not considered an indicator of karyotypic instability.

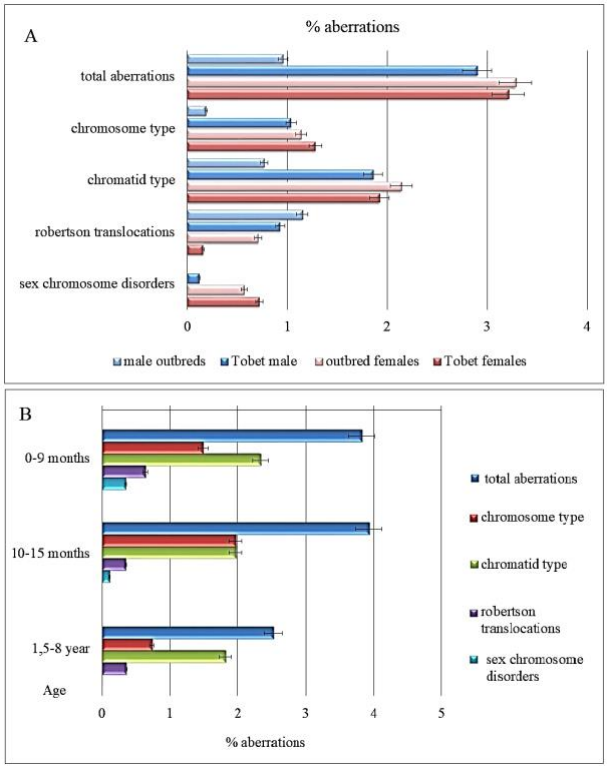

Fig. 4: Frequency of chromosomal abnormalities detected in comparative cytogenetic analysis in Tobets and outbred dogs. A - depending on sex distribution; B - different age groups

Additionally, one male dog exhibited a Multi-Aberrant Cell (MAC) containing multiple breaks, fragments, and exchanges. Generally, MACs occur in response to radiation or toxic exposure in humans or in experimental studies, but they are extremely rare in healthy individuals (Kim et al., 2022).

Assessment of the Adaptive Potential of Tobet Dogs in a Comparative Aspect

The adaptive properties of the body are based on individual and species-specific features of the genetic structure, which are usually activated by stress factors of a certain intensity and duration. To assess adaptation to ionizing radiation and predict radiosensitivity in humans, the ex vivo method of irradiating human peripheral blood lymphocytes with a damaging dose of γ-radiation (1–2 Gy) is used, known as G0-chromosomal radiosensitivity (Baert et al., 2017; Rajaraman et al., 2018; Palumbo et al., 2019). Since Tobet dogs must live and demonstrate their working qualities in a sharply continental climate, they must be characterized by a high level of thermal adaptation, including to high temperatures. By analogy with the method of assessing radiosensitivity in humans, the adaptive capabilities and thermosensitivity of the studied groups of dogs were assessed. For this purpose, experiments were carried out involving ex vivo exposure of their blood lymphocytes to a temperature of 42°C, which is critical for the dog's body. The results of the experiments are presented in Table 2.

The obtained data demonstrate an almost identical frequency of chromosomal abnormalities induced by critical temperature (42°C) in Tobet and outbred dogs. However, some differences in the response of the compared dog groups were observed. In Tobet dogs, the increase in the frequency of CA was minimal, whereas it increased 1.5-fold in outbred dogs ($p \leq 0.05$, $F(1, 28) = 4,32$, $d = 0,39$; $t(28) = 2,09$). That is, when assessing G_0-chromosomal heat sensitivity, Tobet dogs are more resistant to critically elevated temperatures than outbred dogs. The coefficient of variation of CA under the influence of critical temperature was 36% in Tobet dogs and 47% in outbred dogs. The spectrum of detected aberrations did not differ from the spectrum identified in the study of the spontaneous level and predominantly characterized by chromatid-type aberrations.

Table 2: Study of the frequency of chromosomal abnormalities during in vitro exposure of temperature factors on lymphocytes of Tobet and outbred dogs

Variant	Total cells	Total aberrations	Chromo-some type	Chromatid type	RT
Tobet dogs, t 42ºC	2265	3.36±0.38	1.06±0.21	2.30±0.31	0.31±0.12
Tobet dogs, control	2470	3.04±0.34	0.9±0.20	2.15±0.28	0.24±0.01
Outbred dogs, t 42ºC	1660	3.37±0.44	1.2±0.21	2.17±0.35*	1.20±0.3
Outbred dogs, control	1380	2.17±0.37	1.01±0.27	1.16±0.29	0.43±0.17

RT - Robertsonian Translocations
*$p \leq 0.05$ (t-test, ANOVA) difference of values is statistically significant between exposure to the damaging factor and the corresponding control

Discussion

For the first time, a study on the cytogenetic status of the indigenous Kazakh Tobet breed has revealed heteromorphism in chromosome pair 1, as well as heteromorphism and abnormalities associated with the sex chromosomes in some animals. Dogs with a chimeric leukocyte karyotype (78,XX/78,X(Xq-)) and (78,XX/78,XY) were identified, but this had no effect on sexual dimorphism. It is known that in some animal species chimerism of the sex chromosomes is associated with reduced fertility. The development of chimerism is associated with the development of vascular anastomoses and the exchange of blood cells between opposite-sex littermates during fetal development. This phenomenon is common in ruminants (Esteves et al., 2012), but also occurs in other species, including dogs. The different frequency of leukocyte chimerism in different species is probably related to placental type. Cotyledonary placentation (ruminants) or diffuse placentation (pigs, horses) is associated with an increased risk of anastomoses in litters with multiple offspring (Szczerbal et al., 2019). In cats and dogs, the incidence of anastomosis is much lower due to zonary-type placenta. However, larger litter sizes may contribute to a higher incidence of leukocyte chimerism (Szczerbal and Switonski, 2021; Sumner et al., 2021; Switonski et al., 2000).

A comparative assessment of genome instability in Tobet dogs and outbred dogs revealed statistically significant differences in the stability of their genetic apparatus. In addition, the study showed the highest degree of genome stability in a closely related breed, the Central Asian Shepherd dog. These findings indicate a different degree of genetic instability in different dog breeds, with a significantly higher degree of instability observed in purebred dogs. The results are consistent with the findings of (Santovito et al., 2020) who found not only a different degree of cytogenetic abnormalities in various

dog breeds, but also a significant increase in their frequency in purebred dogs compared to outbred dogs (Santovito et al., 2024). The observed level of genetic instability in outbred dogs differs slightly from the data on the spontaneous level of CA in outbred dogs reported by Caliri et al., (2023). In their study, 1.3±0.3% chromatid breaks, and no chromosomal breaks were recorded. In our study, we recorded 1.56±0.25% chromatid breaks and 0.74±0.17% chromosome breaks. It is well known that the level of cytogenetic abnormalities in humans and animals (genetic instability) not only serves as an indicator of genetic status and health, but also reflects the environmental conditions of the region. The observed differences are probably influenced not only by the selection of examinated animals, but also by the ecological conditions of their habitat.

Individual analysis of cytogenetic abnormalities revealed a high percentage of variation in the dogs studied 7% in outbred dogs, 32% in Central Asian Shepherd Dogs, and 60% in Tobet dogs. According to statistical criteria, if the coefficient of variation does not exceed 33%, the population is considered homogeneous, and if it exceeds 33%, it is considered heterogeneous. In other words, only the Central Asian Shepherd dog breed is genetically stable. Such results in outbred dogs can be explained by their genetic diversity. In Tobet dogs, taking into account the small number of this indigenous breed and certain methodological limitations, the increased level of their genetic instability should perhaps be attributed not only to breed characteristics of genetic structure and health status, but also to their increased sensitivity to the effects of exogenous factors compared to mixed-breed dogs. Thirty-five percent of dogs, regardless of breed, had elevated levels of chromosomal abnormalities. A high level of chromosomal abnormalities (1.5–2 times higher than average) was recorded in 23% of Tobet dogs and 10% of outbred dogs. Thus, based on the average values and distribution of individual frequencies of chromosomal abnormalities among groups of dogs, reference values for the frequency of CA were determined. They amounted to ≤3.0% (females and males younger than 1.5 years) and ≤2% (females and males older than 1.5 years) for Tobet dogs. For mixed-breed dogs, they were 0.5–3%, and for Central Asian Shepherd Dogs, they were 2–4%.

A distinctive feature of animals whose autosomes are represented by acrocentric chromosomes is an increased frequency of Robertsonian translocations (centric fusion of two acrocentric chromosomes in the pericentromeric regions, resulting in a single metacentric or submetacentric chromosome). These translocations play a significant role in speciation and are one of the mechanisms of karyotype evolution. It is known that all 38 autosomes in dogs are acrocentric, whereas in the red fox, a representative of the canids, all 16 pairs of autosomes are metacentric. The origin of the eight metacentric chromosomes in foxes is believed to be the result of centric fusions of acrocentrics in the ancestors of canids. The possibly increased level of Robertsonian translocations in outbred dogs ($p \leq 0.05$, $F(1, 56) = 2.82$, $d = 0.2$; $t(56) = 2.03$) indicates active evolutionary and speciation processes. The high level of Robertsonian translocations detected in the leukocytes of one male dog (8%) may lead to an increased risk of such abnormalities in germ cells. This, in turn, increases the likelihood of transmission to offspring as an altered karyotype with corresponding phenotypic and clinical defects. Currently, several cases of RT or centric fusions have been identified in the karyotype of dogs (Mayr et al., 1986; Switonski et al., 2003; Szczerbal et al., 2021). One case of centric fusion was discovered in a outbred dogs terrier with multiple developmental anomalies. In its cells, an additional two-armed chromosome was observed, which was formed by the fusion of a large and a small acrocentric chromosome ($2n = 77$). Similar cases have been described in Miniature Poodles with anatomical defects. However, centric fusions have also been found in phenotypically normal dogs. They have been observed in both heterozygous ($2n = 77$) and homozygous ($2n = 76$) conditions (Klenovitsky et al., 2013). While dogs with RT typically have a normal phenotype, they often suffer from reduced fertility or infertility (Switonski et al., 2003, 2011; Stone et al., 1991).

In addition to investigating the spontaneous level of CA, several other, less researched indicators are currently used to assess genetic instability. These include the assessment of adaptability, differences in radio- and chemoresistance, hormesis and others (Cherednichenko et al., 2024; Zhou et al., 2004). Such responses are thought to be due to genomic alterations, weakened defense mechanisms, and suppression of protein synthesis required for the development and/or stimulation of the adaptive response (Wang et al., 2024). Nowadays, cytogenetic research is increasingly used to evaluate not only the impact of environmental and anthropogenic factors, but also to assess the effects of living conditions on the genetic stability of animals and their ability to maintain it. This is crucial as many diseases arise from mutations and genomic instability. Assessment of the adaptive potential of a breed as well as individual dogs is an important indicator of their ability to maintain genetic stability and activate protective mechanisms in response to various environmental factors and conditions. In human studies, in vitro exposure of lymphocyte cultures to various physical and chemical factors is used to evaluate these parameters (Fornalski et al., 2022; Krishnaja and Sharma., 2008).

The assessment of genome instability in response to heat stress is an important indicator of adaptive potential. Heat stress in livestock, for example, leads to health problems and reduced productivity (Thornton et al, 2022). The main cause of these disorders is probably due to changes in the genetic structure of cells (Girjesh and Akanksha, 2017; Lang et al, 2024). This problem is

particularly relevant for indigenous working dogs in regions with harsh continental climates, where heat tolerance and resilience to abrupt temperature swings is essential to maintain functionality throughout the year. In experiments where cells were exposed in vitro to a critical temperature for the organism (42°C), there was a slight increase in CA in Tobet dogs and a more significant increase (1.5 times higher) in outbred dogs. These results, along with the lower coefficient of variation of CA in Tobets (36%) compared to outbred dogs (47%), indicate that Tobet dogs are less thermolabile and can perform well throughout the year without compromising their performance. Direct studies on the development of chromosomal abnormalities in response to heat stress are extremely limited. For example, studies have shown that sperm DNA fragmentation and aneuploidy of chromosomes 13, 18, 21, X, and Y were significantly increased ($p<0.01$-0.001) in sperm collected in the testes during prolonged in vivo heat exposure compared to control values (Zhang et al., 2018). This is likely since spindle microtubules are very sensitive to temperature during mitosis and meiosis and even small changes in temperature can disrupt their structure, leading to chromosomal abnormalities (Lang et al., 2024; Yurttas et al., 2018).

While the present cytogenetic analysis provides novel insights into the genomic instability and adaptive potential of the Kazakh Tobet breed, the relatively limited sample size and geographic coverage pose significant limitations. Future studies should include larger cohorts from geographically diverse populations to offer a more representative assessment of the breed's genetic and cytogenetic status. Nevertheless, practical implementation of cytogenetic evaluation for Tobets used in breeding is now imperative. Individuals exhibiting karyotype abnormalities and high levels of chromosomal aberrations, along with possibly their parents, should be excluded from breeding (testing should be performed at least three times at regular intervals to rule out the influence of endogenous and exogenous factors). Furthermore, cytogenetic results would benefit from molecular validation using complementary methods such as Fluorescence in Situ Hybridization (FISH), array-based Comparative Genomic Hybridization (aCGH), high-density SNP genotyping, and whole-genome sequencing. Notably, whole-genome sequencing and high-density SNP genotyping have already been utilized to clarify the breed's phylogenetic relationships with other dog breeds. The combined use of these molecular techniques together with cytogenetic analyses holds great promise for providing a comprehensive assessment of the Tobet genome and advancing evidence-based breeding strategies.

Conclusion

Cytogenetic analysis revealed genetic abnormalities in some Tobet dogs without obvious clinical manifestations, which could nevertheless affect the health and quality of their offspring. Differences in genetic stability and adaptive potential to a critical temperature (42°C) were also observed between Tobet dogs and unrelated dogs. Overall, the Tobet breed exhibits greater genomic instability than non-Tobet dogs. Despite certain limitations of our study, which call for further methodological refinement, and the scarcity of research in canine cytogenetics, chromosome analysis holds practical value. It facilitates more objective decisions regarding the acquisition and use of dogs and enables a rapid and accurate assessment of an animal's breeding potential and health at an early age. Long-term genetic monitoring programs will contribute to improving the quality of individual dogs and the breed as a whole.

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Author's Contributions

Oksana Cherednichenko: Conceptualization, methodology, formal analysis, investigation, resources, writing original draft preparation, write review and editing, visualization, supervision, project administration.

Anastassiya Pilyugina validation. formal analysis, investigation, visualization.

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Almira Amirgaliyeva methodology, validation, data curation.

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All authors have read and agreed to the published version of the manuscript.

Ethics

The study was approved by the Bioethics Committee of the RSE with the REM "M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry" (Protocol No. 1, August 18, 2023). The research complies with bioethical standards for studies involving animals and humans, as established by the legislation of the Republic of Kazakhstan and the European Convention on Bioethics.

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