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Short communication

Polymorphism in the promoter region of the tumor necrosis factor-alpha gene in cattle herds naturally infected and uninfected with the Bovine Leukemia Virus

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Abstract

The objective of this study was to describe and compare the genetic structure (TNF-α -position 824) of dairy cattle herds infected and not infected with the bovine leukemia virus (BLV). The results of the present study indicate that BLV-positive herds were characterized by similar genetic structure (TNF-α -824A/G). The genetic equilibrium in these herds was preserved, but a tendency to increased frequency of G/G homozygotes was found. The genetic structure of the healthy herd differed considerably from that of leukemic herds.

Key words: gene, TNF-α, polymorphism, leukosis, lymphocytes, bovine leukemia virus

Introduction

The TNF-α gene has been localized on chromosome BTA 23q22 in the BoLA region. An analysis of nucleotide sequences in several cattle breeds revealed the presence of **s**ingle **n**ucleotide **p**olymorphisms (SNPs) at positions -824, -793, -627 in the 5'-flanking region. SNP at position -824 (GeneBank Acc. No. RS109111281) is a result of A to G substitution (TNF-α -824A/G).

The objective of this study was to describe and compare the genetic structure (TNF-α -824A/G) of dairy cattle herds infected and uninfected with BLV.

Materials and Methods

The study was conducted on a population of 299 Black-and-White cows, aged 3 to 7 years, from three tuberculosis- and brucellosis-free herds. The diagnosis of BLV infections was performed on individuals selected randomly from each herd. The indirect immunofluorescence (IMF) technique was applied as described previously (Kaczmarczyk et al. 2008). TNF-α gene polymorphism at position -824 was determined for the first time using the PCR-RFLP method.

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Primers with the following sequence were used: forward 5' GAG AAA TGG GAC AAC CTC CA 3'; reverse 5' CCA GGA ACT CGC TGA AAC TC 3' (Oligo IBB, Poland).

The following PCR-mixture was applied: 100-150 ng DNA, 10x Taq Buffer with Mg2+ (Biotools, Spain), dNTPs – 2.0 mM each (Epicenter, USA), TNF-α primers – 100 pmol each, 0.8 U Taq DNA Polymerase (Biotools, Spain) and H_2O to 25 μl. The amplification program consisted of initial DNA denaturation (95°C/ 3 min), 35 cycles (94°C/ 30 s, 60°C/ 30 s, 72°C/ 30 s) and final synthesis (72°C/ 5 min). Enzyme Sac I identifies sequence 5' GAGCT↓C 3'(allele A) and cuts the amplified gene fragment into 168 bp and 81 bp bands. The A→G mutation (allele G) eliminates the restriction site for Sac I, therefore, the electrophoregram shows a DNA fragment whose size corresponds to the PCR product (249 bp).

Genotype and allele frequencies were calculated, and the Hardy-Weinberg equilibrium was verified by the χ^2 test.

Results and Discussion

Based on the diagnosis of BLV infection, herds A and C were classified as BLV-positive, and herd B – as BLV-negative. The analysis of TNF-α -824A/G gene polymorphism revealed the presence of three genotypes (Fig. 1). Heterozygotes were identified most frequently (Table 1). G/G homozygotes were observed more frequently than A/A homozygotes in both BLV-positive herds, whereas their frequency was considerably lower in the BLV-negative herd (B) compared with BLV-positive herds A and C. The frequency of the mutated allele (-824G) was similar in all herds (Table 1).

The analysis of genetic equilibrium in each herd revealed high conformity between the observed and the expected number of TNF-α genotypes in herd C ($\chi^2 = 0.64$, $p < 0.75$) and small, insignificant differences in herd A ($\chi^2 = 2.12$, $p < 0.35$) (Table 1). Statistically significant differences ($\chi^2 = 12.00$, $p < 0.01$) were observed in herd B where the predominance of heterozygotes and a shortage of both homozygotes was found. Significant differences in genotype frequency were also reported between each of the infected herds and the uninfected herd (Table 1).

The polymorphism of the TNF-α – 824A/G gene and its correlation with BLV infections have been studied only by Konnai et al. (2006). The cited authors also observed differences in genotype frequency between cattle herds infected and uninfected with BLV. A/G TNF-α -824 heterozygotes were more frequently reported in the group of BLV-negative than in BLV-positive cows, but the differences observed were statistically insignificant. Significant variations in genotype frequency were found between individuals at the early (aleukemic leukosis, AL) and advanced (lymphosarcoma, LS) stages of EBL. The differences observed were attributed to a significant increase in the frequency of the G/G genotype and a decrease in the frequency of A/G heterozygotes in cattle with LS (Konnai et al. 2006). The relationship between TNF-α gene polymorphism in the promoter/enhancer region and susceptibility to lymphoproliferative disorders have been also reported in humans infected with HTLV-I (human T-cell lymphotropic virus type 1).

The results of the present study indicate that BLV-positive herds were characterized by similar genetic structure (TNF-α -824A/G). The genetic equilibrium in these herds was preserved, but a tendency to

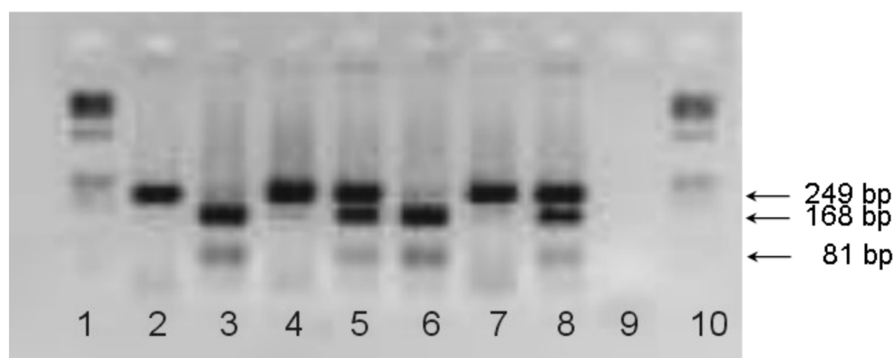

Fig. 1. TNF-α – 824 genotypes in the animals studied.
Lanes 1, 10 – φ X174/Hae III molecular marker; lane 2 – PCR product (249bp); lanes: 3, 6 – A/A (168 bp, 81 bp); 4, 7 – G/G (249 bp); 5, 8 – A/G (249 bp, 168 bp, 81 bp)

Table 1. Distribution of TNF-α -824A/G polymorphism in the cattle herds studied.

Herds	No. of animals	Genotype frequencies			Allele frequencies		*Value χ^2
		AA	AG	GG	A	G	
A[a]	94	0.20	0.57	0.22	0.49	0.51	2.12
B[ab]	48	0.13	0.75	0.13	0.50	0.50	12.00**
C[b]	157	0.17	0.52	0.31	0.43	0.57	0.64
Total	299	0.17	0.58	0.25	0.47	0.53	0.00

* for Hardy-Weinberg equilibrium; ** difference statistically significant at $p < 0.01$; [a], [b] – statistically significant differences at $p < 0.01$ are marked with the same letter

increased frequency of G/G homozygotes was observed. The genetic structure of the healthy herd differed considerably from that of leukemic herds.

Acknowledgments

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