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ARYLESTERASE POLYMORPHISM IN TWO TYPES OF PRAMENKA SHEEP

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The variability of arylesterase (EsA) in the plasma of sheep is an example of the physiological implication of enzyme polymorphism. It was clinically observed that administration of the antiparasitic medicine Haloxane, besides antiparasitic effects, can sometimes induce neurotoxic symptoms and death of the sheep. A reduced activity of arylesterase was determined in all Haloxane succeptioble individuals.

In this paper, the activity of arylesterase is presented for two types of Pramenka sheep. The quick tuba-test identified two phenotypes of the enzyme EsA^+ and EsA^- (Tucker, 1967). The characteristic frequences of EsA^+ and EsA^- genes were 0.021 and 0.79, for Zetska žuja and 0.18, 0.82 in the Svrljiška type of Pramenka. The relavitely high frequency of the EsA^+ gene of Pramenka enables better detoxication of the organophosphorous component than observed in most other tested breeds of sheep. The detemined biochemical polymorphism of the arylesterase locus in Pramenka sheep can be applied alone or together with other genetic markers for identification and (parentage control) of individuals.

Key words: sheep, protein polymporphism, arylesterase

INTRODUCTION

It has been confirmed in practice that Haloxane, an antiparasitic drug, effectively kills nematodes in sheep, but sometimes can induce neurological disturbances and death of the animal. It was observed °TV4°TV4ty was more frequent in some breeds than others. Moreover, Haloxane succeptibility is closely related to the arylesterase (EsA) activity in the plasma of sheep (Lee, 1966).

Evaluation of the plasma arylesterase activity in sheep showed that two types of the enzyme existed (Lee, 1964). Individuals with high esterase activity towards di (2-chloroethyl) aryl phosphates were named Halon-high type and those with low activity levels were classified as Halon-low type. Later , it was confirmed that the two established types were equivalent to the EsA^+ (positive) and EsA^- (negative) types reported by Tucker et al. (1967). The classification

into EsA + and EsA- types was based on the ability of aryilesterase to hydrolyse alpha naphthyl acetate. The sheep with impaired arylesterase activity towards alpha-naphthyl acetate cannot hydrolyse the organophosphate group of Haloxane and they are born susceptible to the drug.

The polymorphism of arylesterase in the plasma of sheep is very complicated. Application of different substrates (indophenyl acetate, alpha-naphthyl acetate, beta-naphthyl acetate) and/or different analytical tecnica (electrophoresis, spectrophotometry) revealed that three different phenotypes can be identified within each of the activity types. Electrophoresis enabled identification of 4 out of 6 phenotypes. The main characteristic of the electrophoretic paltern of EsA types is not the different mobility, but the different staining characteristics (Lee, 1964; Gahn, and Görannson, 1970). Arylesterase activity in sheep plasma is controlled by the dominant autosomal allele EsA+, while the absence of activity is related to the product of the EsA-gene, which is recessive (Tucker et al., 1967). Arylesterase activity cannot be detected in lambs during the first perinatal week. The peak of enzyme activity occurs when the lamb is two to three months old. These age-dependent variations must be considered when the variability of the enzyme is applied in identification of the animal (Tucker, et al. 1967).

Since arylesterase plays an important metabolic role seen in clinical disturbances due to impaired activity of the enzyme, an evaluation of the polymorphism of the arylesterase locus in two types of Pramenka sheep is presented in this paper.

MATERIAL AND METHODS

A total of 90 blood samples obtained from two types of Pramenka sheep were analyzed. Among these 40 animals belonged to the Zetska Žuja and 50 to the Svrljiška type of Pramenka sheep.

The tuba-test developed by Tucker et al. (1967) for esterases in sheep was used for determination of arylesterase activity in the serum. This test involves a colour change when plasma is mixed with alpha-naphthyl acetate and Fast blue BB salt. Within seconds EsA+ samples developed a deep yellow-brown coloration, whereas the negatives turned dark green after standing for 30 or more seconds. The results of the quick tuba test were in complete agreement with the zymogram method for diagnosing EsA+ and EsA- phenotypes.

RESULTS

The evaluation of arylesterase polymorphism in Zetska žuja and Svrljiška types of Pramenka sheep revealed that two phenotypes of the enzyme (EsA+ and EsA-) were distributed in the tested populations.

According to the laboratory data for phenotype the frequencies of the two different EsA genes were calculated. The frequencies of these EsA genes in the two types of Pramenka sheep are presented in table 1.

Table 1. Gene frequencies at arylesterase locus in two types of Pramenka

Tupe	No		EsA phenotypes		X^2	Gene frequency	
			EsA+	EsA-		EsA+	EsA-
Zetska žuja	40	Exp	14,73	25.37	0.009	0.21	0.79
		Obs	15	25			
Svrljiška	50	Exp	16.38	33.62	0.012	0.18	0.82
		Obs	16	34			
					p 0.05		

The frequencies of EsA+ and EsA- types were similar in Zetska žuja and Svrljiška sheep. A high frequency of the EsA- gene was a characteristic of both Pramenka types. A similar distribution has been reported in other sheep breeds.

DISSCUSSION

The literatury data showed that the frequencies of the EsA+ gene are within certain limits, ranging from 0.00 in Merino, Soay and Racka (Tucker, 1975) to 0.49 in Friesan sheep (Buis and Tucker, 1983). A very low frequency of EsA+ alleles (under 0.10) is a characteristic of nearly all tested breeds: Dorset, Southdown (Tucker, et al. 1967), Herdwick (Lee, 1964) Suffolk (Tucker, 1967), Tasmanian merino (Tucker, 1975), Black Blaze, Scodnebeker (Buis and Tucker 1983), etc. Thus, the frequency of the EsA+ qene in the tested types of Pramenka is relatively high compared to the distribution of the gene reported in the other breeds. The most similar breeds to Pramenka are: Navajo (0.18) (Tucker, et al. 1967), Hungarian merino (0.19) (Fesus 1972), Finish landrace (0.19), (Tucker, 1975), Kent (0.21) (Lee, 1964), Awassi (0.22) (Tucker, 1975) From the data in the literature, only Ramboullet (0.36) (Tucker, et. al. 1967) and Friesan sheep (0.49) (Buis and Tucker, 1983) are characterized with a higher EsA+ gene frequency than Pramenka. The relatively high frequency of the EsA+ gene in these types of Pramenka enables more animals to detoxicate the organophosphorous component adequately than observed in most other tested breeds of sheep.

The determined polymorphism of plasma arylesterase in Zetska Žuja and Svrljiška types of Pramerka breed and the quick determination of the phenotypes enables easy application of the arylesterase polymorphism test in parentage control and identification in the breed.

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POLIMORFIZAM ARILESTERAZE KOD DVA SOJA PRAMENKE

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SADRŽAJ

Polimorfizam arilesteraze (EsA) u plazmi ovaca predstavlja jedan od primera fizioloških implikacija polimorfizma enzima. Praksa je pokazala da upotreba antihelmintika Haloksana dovodi do uginuća nematoda ali da u nekim slučajevima može uzrokovati paralizu i smrt jedinki, Kod svih ovih ovaca utvrđena je smanjena aktivnost arilesteraze.

U ovom radu ispitivana je aktivnost arilesteraze u plazmi dva soja ovaca rase Pramenka. Korišćenjem tuba testa (Tucker, 1967) utvrđena su dva fenotipa ovog enzima: EsA+ i EsA- u plazmi ovaca. Karakteristična frekfenca EsA^+ i EsA^- gena u ispitivanim populacijama bila je (0.21 i 0.79) i (0.18 i 0.82). Relativno visoka učestalost EsA+ gena kod sojeva pramenke pruža im mogućnost bolje detoksikacije organofosforne komponente antihelmintika, od većine do sada ispitanih rasa. Utvrđeni biohemijski polimorfizam arilesteraze u plazmi pramenke može, uz druge markere naći primenu u identifikaciji i kontroli porekla jedinke.