Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

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To cite this article: Gökselin Camoglu & Cengiz Elmaci (2005) Carbonic Anhydrase (Ca) and X-Protein(X-p) Types in Turkish Sheep Breeds, Journal of Applied Animal Research, 28:2, 125-128, DOI: 10.1080/09712119.2005.9706806

To link to this article: https://doi.org/10.1080/09712119.2005.9706806

J. Appl. Anim. Res. 28 (2005) : 125-128

Carbonic Anhydrase (Ca) and X-Protein(X-p) Types in Turkish Sheep Breeds*

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(Received November 29, 2004; accepted August 2, 2005)

Abstract

Camoglu, G. and Elmaci, C. 2005. Carbonic anhydrase (Ca) and X-protein (X-p) types in Turkish sheep breeds. J. Appl. Anim. Res., 28: 125-128.

Carbonic anhydrase and X-protein types were analyzed by the method of starch gel electrophoresis in a total of 197 blood samples collected from both sexes of three Turkish sheep breeds. Carbonic anhydrase and X-protein loci were polymorphic in all breeds. The gene frequencies ranged from 0.881 to 0.974 and from 0.805 to 0.884 for CaS and x, respectively. Further, a very good agreement was found between the observed and expected carbonic anhydrase phenotypes.

Key words: Carbonic anhydrase, X-protein, genetic polymorphism, sheep.

Introduction

Polymorphic blood traits are very useful for studies of evolution, relationship and genetic structure of breeds. In addition, they can also be used for kinship analysis as breed markers, a function of great importance, especially for the preservation of breeds (Igarashi, 2000). The biochemical polymorphisms of sheep consist of variation in the proteins of blood plasma/serum, red cells, white cells and milk. So far numerous proteins showing variations have been identified by different workers. Besides, there are a number of the studies in which blood protein polymorphism was demonstrated among different Turkish sheep breeds (Asal *et al.*, 1996; Dellal *et al.*, 1997; Elmaci and Uzatıcı, 2002; Kargin *et al.*, 2003), but there is no information on carbonic anhydrase and X-protein polymorphism in Turkish sheep breeds.

The first report on variants in sheep carbonic anhydrase and X-proteins were made by Tucker *et al.* (1967). There after a number of workers have conducted similar studies on various sheep breeds (Ordas and Primitivo, 1983; Zanotti Casati *et al.*, 1988; 1990; Osfori and Fesüs, 1996; Missohou *et al.*, 1999). But no work appears to have been done on Kıvırcık, Gökçeada and Tahirova breeds. The objective of the present study was to determine the carbonic anhydrase and X-protein types in these three Turkish sheep breed populations.

* This study is a part of the first author's MSc thesis.

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J. Appl. Anim. Res. 0971-2119/2005/$5.00

Materials and Methods

One hundred ninety seven blood samples were collected from three Turkish sheep breeds from Canakkale province of Turkey namely Kıvırcık (38), Gökçeada(54) and Tahirova (105).

Kıvırcık is a medium sized fine tailed native sheep breed raised in north-western part of Turkey. It is farmed for meat, wool and milk, and is well adapted to cool climate.

Gökçeada is the smallest sized fine tailed native sheep breed raised in Gökçeada Island and Çanakkale province of Turkey. It is also reared for milk, meat and wool, and is well adapted to cool climate.

Tahirova is a medium size crossbred (East Frisian x Kıvırcık) farmed for milk and wool in the area around the Ege and Marmara region of Turkey. This breed is well adapted to step climate.

About 10 ml of venous blood was collected in test tubes containing EDTA as anticoagulant. Blood samples were separated by centrifugation into plasma and red cells. Red cells were washed and stored at -20 C prior to electrophoresis. The samples were subjected to horizontal starch-gel electrophoresis according to method described by Tucker *et al.* (1967). The gels were stained with amido black 10B.

Gene frequencies for polymorphic traits were computed by the direct gene counting method for the carbonic anhydrase locus and by taking the square root of the frequencies of the recessive homozygotes for X-protein loci assuming genetic equilibrium. Adherence to Hardy-Weinberg equilibrium (HWE) was checked using the $\chi 2$ method.

Results and Discussion

We have obtained gene frequency data for carbonic anhydrase and X-protein loci in these three sheep breeds of Turkey for the first time. As shown in Fig. 1 two main regions are visible in addition to haemoglobin. One of them cathodally placed protein is carbonic anhydrase and second anodally migrating region is X-protein.

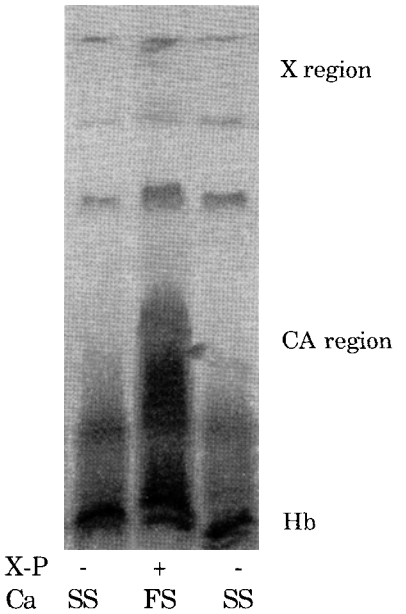

Fig. 1: Red cell carbonic anhydrase and X-protein phenotypes.

Almost all sheep possess one single slow band designated as CaS, a rare faster variant CaF and heterozygous sheep have both bands (CaFS). Two phenotypes of carbonic anhydrase, which were designated FS and SS, were found in this study. The Ca locus had no FF genotype but the most common genotype at this locus was SS. FS heterozygosity occurred at a very low frequency while CaF allele of the highest frequency was observed in the Tahirova breed. Allele frequencies were calculated, assuming co-dominant inheritance of the alleles CaF and CaS (Table 1). The allelic constitution was generally similar in the three breeds. Deviations from HWE were not significant ($P > 0.05$) at the carbonic anhydrase locus for all breeds. However, in different sheep breeds, three co-dominant carbonic anhydrase alleles CaF, CaS and CaM

Table 1
Phenotype distribution and mean gene frequencies of carbonic anhydrase and X-protein loci in studied Turkish sheep breeds

Breed	No. of sheep	Carbonic anhydrase						X- protein			
		Phenotypes*			Gene frequencies		Test of	Phenotypes		Gene frequencies**	
		FF	FS	SS	CaF	CaS	HWE	X(+)	X(-)	X	x
Kıvırcık	38	- / 0.026	2 / 1.925	36 / 36.049	0.026	0.974	$\chi^2 = 0.029$ NS	9	29	0.126	0.874
Gökçeada	54	- / 0.169	6 / 5.710	48 / 48.121	0.056	0.944	$\chi^2 = 0.184$ NS	19	35	0.195	0.805
Tahirova	105	- / 1.487	25 / 22.016	80 / 81.497	0.119	0.881	$\chi^2 = 1.891$ NS	23	82	0.116	0.884

*Upper row; observed values, lower row expected values. HWE: Hardy-weinberg equilibrium. NS: Not significant.
** Calculated by the assumption of Hardy-weinberg equilibrium.

have been described, the CaS allele being more frequent. The frequencies of CaS allele in the three breeds in the present study were similar to those reported earlier (Tucker *et al.*, 1967; Zanotti Casati *et al.*, 1990; Morera *et al.*, 1993). CaM allele was observed only in few sheep breeds such as Churra, Manchega (Ordas and Primitivo, 1983), Varesina (Zanotti Casati *et al.*, 1988), Djallonke, Fulani and Touabire (Missohou *et al.*, 1999) with generally low frequencies.

The variation of X-protein observed (Fig. 1) in the present study were similar to those detected in different sheep breeds (Osfori and Fesüs, 1996; Missohou *et al.*, 1999; Tsunoda and Sato, 2001). X-protein locus was found to be polymorphic in the sheep breeds investigated. Two phenotypes of X-protein (X-positive and X-negative) were observed. The allelic constitution was similar in the three breeds (Table 1). The negative phenotype was predominant in all sheep breeds, whereas, the highest frequency of negative type was found in the Tahirova breed. The allelic frequency for x was high and the same tendency was also found in the different sheep breeds (Tucker *et al.*, 1967; Osfori and Fesüs, 1996; Missohou *et al.*, 1999; Tsunoda and Sato, 2001). The phenotypic distribution of X-protein system in different sheep breeds were compared using gene frequencies data for X-protein locus. The results showed that there was no difference between Turkish sheep and other sheep population with respect to X-protein system. More work on these lines is desired.

Acknowledgements

We are grateful to Dr. Kenji Tsunoda (Department of Legal Medicine, Showa University School of Medicine, Tokyo 142-8555, Japan) for providing reference blood samples and revision of the manuscript. This work was supported by a grant of the Scientific Research Projects Council of Uludag University (Project number 2003-89), Bursa, Turkey.

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जी. कैमोग्लु, सी. एल्मासी। टर्की भेड़ों की नस्लों में कार्बोनिक एन्हाइड्रेज (सीए) और एक्स-प्रोटीन (एक्सपी) के प्रारूप।

टर्की की भेड़ों की तीन नस्लों के दोनों लिंगों से रूधिर के कुल 197 नमूनों का स्टार्च जेल इलेक्ट्रोफोरेसिस विधि से कार्बोनिक एन्हाइड्रेज और एक्स-प्रोटीन प्रकारों में विश्लेषण किया गया। सभी नस्लों में कार्बोनिक एन्हाइड्रेज और एक्स-प्रोटीन लोसाई बहुरूपी थे। कार्बोनिक एन्हाइड्रेज और एक्स-प्रोटीन के लिए जीनों की बारम्बारता क्रमशः 0.881 और 0.974 थी। इसके अतिरिक्त कार्बोनिक एन्हाइड्रेज के प्रेक्षित और प्रत्याशित समलक्षणों में बहुत अच्छा समेल पाया गया।