MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (4): 630-633, 2009
ISSN: 1680-5593


# Determination of Adenosine Deaminase and Acid Alpha Naphthyl Acetate Esterase Enzyme Activity of Kilis Goats

[1]I.S. Harem, [2]E. Karadag Sari, [1]M. Kocak Harem and [3]E. Atakisi
[1]Department of Histology and Embryology,
Faculty of Veterinary Medicine, Harran University, 63300, Sanliurfa, Turkey
[2]Department of Histology and Embryology, [3]Department of Biochemistry,
Faculty of Veterinary Medicine, Kafkas University, 36100, Kars, Turkey

**Abstract:** We examined alpha naphthyl acetate esterase and adenosine deaminase enzyme activity in peripheral blood of kilis goat. Our aim was to determine the percentage of ANAE positive lymphocytes and Adenosine Deaminase (ADA) measurements. The proportion of ANAE positive lymphocytes was determined to be 66%, ADA activity was determined to be 15.0±1.15 U $L^{-1}$. While, T lymphocytes showed an ANAE positive reaction, the eosinophil granulocytes, neutrophil granulocytes and monocytes also, showed a positive reaction. The reaction observed in T lymphocytes was a red-brown coloration, usually 1-2 granules, but enough small granules to fill the cytoplasm were detected rarely.

**Key words:** ADA, ANAE, peripheral blood, kilis goat

## INTRODUCTION

Alpha Naphthyl Acetate Esterase (ANAE) is a lysosomal enzyme that is active in different maturation phases of T lymphocytes and it participates in cytotoxic effects. Its existence can be detected by an ANAE staining method and T lymphocytes can be distinguished in peripheral blood (Mueller *et al*., 1975; Higgy *et al*., 1977). Determination of the ratio of peripheral blood T and B lymphocytes in healthy animals may assist the diagnosis of some diseases. The ANAE positive lymphocyte proportion for healthy cattle is 47.7%, while, the proportion decreases to 7.3% in cases of enzootic bovine leukocytosis (Basso *et al*., 1980; Kajikawa *et al*., 1983; Thurmond *et al*., 1990).

Adenosine deaminase (ADA; EC 3.5.4.4), an amino-hydrolase, is the key enzyme of purine metabolism. It controls levels of adenosine and deoxy-adenosine in the cell (Aktepe *et al*., 1999). Most important physiological role of ADA is related with lymphocyte differentiation and proliferation (Baganha *et al*., 1990). Hereditary deficiency of this enzyme with high activity in T lymphocytes causes functional disturbance in T and B lymphocytes and severe combined immune deficiency syndrome (Franco *et al*., 1990; Alan *et al*., 2002). It has been reported that the decrease in ADA levels were related to immune system deficiency caused by numbered and functional T lymphocyte defect and thus, ADA activity may be used as a celluler immunity indicator (Alan *et al*., 2002; Hatipoglu *et al*., 2003). Positive relationship between ADA level and T lymphocyte ratio in some human disease with pleural effusion has been reported by Chopra *et al*. (1988). However, Yilmaz *et al*. (1991) do not agree with this opinion.

Kilis goat is one of the best-known dairy goat breeds in Turkey. These goats in the Southeast Anatolia region are well adapted to the extreme climatic conditions of Turkey (Iriadam, 2004). The objective of this study was to determine the percentage of T lymphocyte and ADA values in peripheral blood of healthy kilis goats.

## MATERIALS AND METHODS

Heparinized blood samples were taken from 10 healty, 1-2 years of age, pure-bred kilis goats, which body weight of 35.0±10.0 kg.

**For ANAE activity:** Blood smears were prepared and air dried for ANAE staining. They were then fixed in glutaraldehyde-acetone solution for 3 min at -10°C, rinsed in distilled water and air dried. Air dried slides were incubated for 3 h at room temperature in a mixture of 40 mL 0.067 M phosphate buffer, pH 5.0, 2.4 mL hexazotized pararosaniline and 10 mg α-naphthyl acetate

**Corresponding Author:** Ismail Sah Harem, Department of Histology and Embryology, Faculty of Veterinary Medicine, Harran University, 63300, Sanliurfa, Turkey

(Sigma Chemical Co., St. Louis, MO, N8505) in 0.4 mL acetone, pH 5.8 after adjustment with 2 N NaOH. Hexazotized pararosaniline was prepared by combining equal volumes of 2 solutions. The first solution consisted of 1g pararosaniline (Sigma, P 3750) in 20 mL distilled water with 5 mL 12 N HCl, heated gently, cooled to room temperature and filtered. This solution stored in a dark bottle as stock solution. The 2nd solution was a freshly prepared 4% aqueous solution of sodium nitrite. Following incubation, the slides were washed with distilled water, counterstained with methylene blue for 10 min, rinsed in distilled water, dehydrated in increasing concentrations of ethanol, cleared in xylene and mounted with permount.

After ANAE enzyme staining, the slides were examined by light microscopy. The lymphocytes with typical reddish brown granules were considered positive. The positive lymphocytes proportions in every smear were determined by counting 300 lymphocytes in 3 areas (100/field, 10×40 magnification).

**For analyses of ADA activity**: Blood samples were centrifuged 3000 rpm and 15 min. Plasma were separated and stored -20° C until to be analyzed. Plasma ADA activity was carried out according to the method reported by Giusti and Galanti (1984). According to the this method, Adenosine, which is a substrat of ADA is incubated at 37° C, 1 h with plasma for ammonia formation. Ammonia causes strong blue coloured indophenol formation with sodium hypochloride and phenol in alkaline solution. Ammonia concentration is linear with absorbance of indophenol. Sodium nitroprusside shows catalyst effect in this reaction. Blue coloured indophenol absorbance is read at 625 nm.

ADA levels are calculated according to formule as a U $L^{-1}$:

$$ADA\ (U\ L^{-1}): A/B \times 50 = \mu mol/min/L$$

A = Absorbance of sample-absorbance of sample blank

B = Absorbance of standard-absorbance of reactive blank

50 = A factor, which take from dilution factor and standard concentration

**Statistical analyses**: Mean and standard deviation of value was done by using microsoft excel programme.

## RESULTS

ANAE enzyme staining detected positive reactions in T lymphocytes, monocytes, eosinophil granulocytes and neutrophil granulocytes from kilis goat peripheral blood leukocytes and negative reactions in platelets and B lymphocytes.

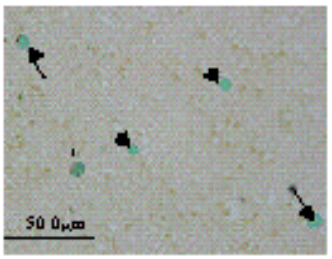


Fig. 1: ANAE staining in kilis goat. arrows: ANAE-positive T lymphocyte, arrow heads: ANAE negative B lymphocyte, asterisk: a neutrophil granulocyte show granular positivity

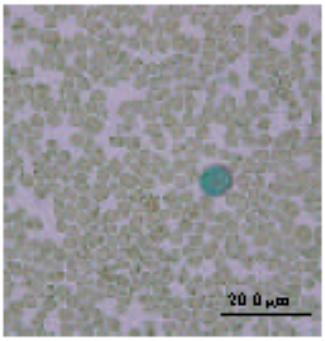


Fig. 2: ANAE staining in kilis goat: diffuse small granules in T lymphocytes

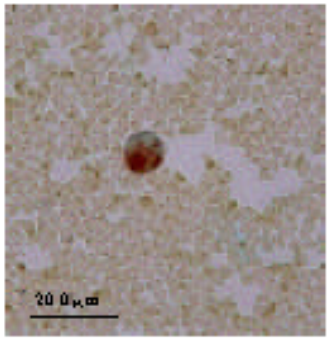


Fig. 3: Diffuse granular ANAE-positive staining in monocytes of kilis goat

ANAE stained T lymphocytes show either 1-2 red-brown large or small granules (Fig. 1). Monocytes (Fig. 2) and eosinophil granulocytes show strong, diffuse granular positivity (Fig. 3). Neutrophil granulocytes show granular positivity (Fig. 4). As a result of ANAE enzyme staining, the proportion of positively stained T lymphocytes in peripheral blood was 66% and the proportion of B lymphocytes was 34%.

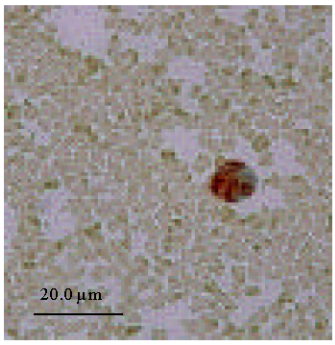


Fig. 4: ANAE staining in the eosinophil granulocyte of kilis goat

Plasma ADA activities of kilis goats (n = 10) were also found average 15.0±1.15 U $L^{-1}$.

## DISCUSSION

ANAE activity of peripheral blood leucocytes in different animal species (Mueller *et al*., 1975; Asti *et al*., 1996) and human (Higgy *et al*., 1977) change due to stain pH and staining duration. Also, the best reaction in goat has been obtained with 3 h staining at pH value of 5.8 (Asti *et al*., 1996). For this reason, the same pH value and staining duration was used in the current study.

T lymphocytes and also monocytes, eosinophyle and neutrophyle granulocytes had positive reaction to ANAE in the peripheral blood of goats (Kurtdede *et al*., 1995; Asti *et al*., 1996). It has been emphasized that the number of T lymphocytes had large granules (n = 1-2) and granular positivity, observed in neutrophyle granulocytes was strong and diffuse in monocytes and eosinophyle granulocytes. It has also, been mentioned that no reaction in B lymphocytes and platelets (Kurtdede *et al*., 1995; Asti *et al*., 1996). Osbaldiston and Sullivan (1978) determined positive reaction in the neutrophyle granulocytes of goats, whereas no reaction in the eosinophyle granulocytes. Our data is in parallel to findings of Asti *et al*. (1996) and Kurtdede *et al*. (1995). However, regarding with eosinophyle granulocytes, no similarity to that of Osbaldiston and Sullivan (1978). In the present study, stained small amounts of T lymphocytes as large quantities of small granules was also observed.

It has been reported that the proportions of ANAE positive peripheral blood T lymphocytes is 63.6% in Angora goats (Kurtdede *et al*., 1995) and also, 63% in cattle, close species to goat (Paul *et al*., 1979). We found the proportion of ANAE-positive lymphocytes in kilis goat peripheral blood to be 66%.

In this study, ADA activities of kilis goat plasma were found as 15.0±1.15 U $L^{-1}$. Only Rodrigues *et al*. (2000) has reported that ADA activity was 19.5±6.0 U $L^{-1}$. Both species and region and climate differences may explain numerical differences with our and other study (Rodrigues *et al*., 2000) results. It has not found any literature data in this subject in the goat. However, it has been reported that highest value of ADA in the sheep, which is a nearest animal for comparison to goat as 10.51±0.47 U $L^{-1}$ (Alan *et al*., 2002).

ADA activity (Rodrigues *et al*., 2000) and T lymphocyte ratio (Basso *et al*., 1980; Kajikawa *et al*., 1983) are used as a marker to determine some infectious diseases. Caprine Arthritis-Encephalitis (CAE) is widespread and persistent infection during animal life. This disease has been reported to cause lesions at many organs, including thymus (Al-Ani and Vestweber, 1984). This study is the first report on ADA and T lymphocyte ratio values of healthy kilis goats. Diagnostic efficiacy of these values as a marker in animals infected with CAE should be investigated.

## REFERENCES

Aktepe, N., S. Avci., O. Erel and A. Kocyigit, 1999. Serum adenosine deaminase, guanase activities and uric acid concentrations in patients with Vivax Malaria, Turk. J. Biochem., 1 (24): 26-29. http://www. turkjbiochem.com/1999/1_026_029.pdf.

Al-Ani, F.K. and J.G.E. Vestweber, 1984. Caprine Arthritis Encephalitis syndrome (CAE): A review. Vet. Res. Comm., 8: (4) 243-253. PMID: 6097020.

Alan, M., Z.T. Agaoglu, A. Uyar and N. Altug, 2002. Serum adenosine deaminase levels in different periods of pregnancy in Ewes. Truk. J. Vet. Anim. Sci., 26: 487-490. http://journals.tuitak.gov.tr/veterinary/issues/vet-02-026-3/vet-26-3-9-0011-29.pdf.

Asti, R.N., B. Alabay, N. Kurtdede, H. Altunay and L. Ergun, 1996. Determination of acid alpha-naphthyl acetate esterase enzyme activity in peripheral blood leucocytes of various animals species. Vet. J. Ankara Univ., 43: 129-133. http://apps.isiknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=1&SID=V1ei1aI@M1PL@m3plN4&page=3&doc=21&colname=CABI.

Baganha, M.F., A. Pego, M.A. Lima, E.U. Gaspar and B. Pharma, 1990. Serum and pleural adenosine deaminase correlation with lymphocytic populations, Chest., 97 (3): 605-610. PMID: 1689629.

Basso, G., M.G. Cocito, G. Semenzato, A. Pezzutto and L. Zanesco, 1980. Cytochemical study of thymocytes and T lymphocytes. Br. J. Haematol., 44: 577-582. DOI: 10.1111/.1365-2141.1980.tb08712.x.

Chopra, R.K., V. Singh, H. Lal, A.S. Saini, R.K. Chawla and B. Raj, 1988. Adenosine deaminase and T-lymphocyte levels in patients with pleural effusions. Ind. J. Tub., 35 (1): 22-24. http://mohw.nic.in/lrs/IJTB/Year%201988/january%201988.pdf#page=23.

Franco, R., J.M. Aran, D. Colomer, E. Matutes and J.L. Vives-Corrons, 1990. Association of adenosine deaminase with erythrocyte and platelet plasma membrane: An immunological study using light and electron microscopy. J. Histochem. Cytochem., 38: 653-658. PMID: 2332624.

Giusti, G. and B. Galanti, 1984. Colorimetric Method: Methods of Enzymatic Analysis. 3rd Edn. Weinheim, Verlac Chemie, 315-323. ISBN: 3-52728045-5.

Hatipoglu, K., I. Yuksekol, M. Ozkan, A. Balkan, E. Tozkoparan, I. Bedirhan, H. Bilgic and N. Demirci, 2003. The importance of measuring serum adenosine deaminase in tuberculosis. Gulhane Med. J., 45 (2): 165-168. http://www.gulhanemedicaljournal.org/pdf/pdf-GMJ-59.pdf.

Higgy, K.E., G.F. Burns and G.J. Hayhoe, 1977. Discrimination of B, T and null lymphocytes by esterase cytochemistry. Scand. J. Haematol., 18: 437-448. PMID: 327533.

Iriadam, M., 2004. Some haematological and biochemical parameters in kilis goat. Vet. J. Ankara Univ., 51 (2): 83-85. http://www.ankara.edu.tr/kutuphane/Veteriner_Web_HTML /veteriner2004_2.htm.

Kurtdede, N., R.N. Asti, H. Altunay and C. Akdeniz, 1995. Use of alpha-naphtyl acetate esterase and surface immunglobulin staining to identify T and B lymphocytes in Angora goats. Vet. J. Ankara Univ., 42: 85-90. http://apps.isiknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=4&SID=V1ei1aI@M1PL@m3plN4&page=3&doc=26&colname=CABI.

Kajikawa, O., H. Koyama, T. Yoshikawa, S. Tsubaki, H. Saito, 1983. Use of alpha-naphthyl acetate esterase staining to identify T lymphocytes in cattle. Am. J. Vet. Res., 44: 1549-1552. PMID: 6194722.

Mueller, J., G. Brun del Re, H. Buerki, H.U. Keller, M.W. Hess and H. Cottier, 1975. Nonspesific acid esterase activity: A criterion for differentiation of T and B lymphocytes in mouse lymph nodes. Eur. J. Immunol., 5: 270-274. DOI: 10.1002/eji.18300 50411.

Osbaldiston, G.W. and R.J. Sullivan, 1978. Cytochemical demonstration of esterases in peripheral blood leukocytes. Am. J. Vet. Res., 39: 683-685. PMID: 25603.

Paul, P.S., D.R. Senogles, C.C. Muscoplat and D.W. Johnson, 1979. Enumeration of T cells, B cells and monocytes in the peripheral blood of normal and lymphocytotic cattle. Clin. Exp. Immunol., 35: 306-316. PMID: 312176.

Rodrigues, L.F.S., G.H. Freire and M.R. Vale, 2000. Multiple iso-forms of caprine adenosine deaminase. Isr. J. Vet. Med. 55 (4): 134-137. http://isrvma.org/article/55_4_4.htm.

Thurmond, M.C., R.L. Carter, J.P. Picanso and K. Stralka, 1990. Upper-normal prediction limits of lymphocyte counts for cattle not infected with bovine leukemia virus. Am. J. Vet. Res., 51: 466-470. PMID: 2156474.

Yilmaz, T., A. Saltik, K. Topal and E. Cakir, 1991. Adenosine deaminase measurements and T-lymphocyte counts in the blood and pleural fluid of malignant and TBC pleurisy patients. J. Islamic Acad. Sci., 4 (1): 66-70. http://www.medicaljournal-ias.org/4_1/Yilmaz.pdf.