

Total serum IgG, IgM, IgA and IgE immunoglobulin concentrations in purebred Anatolian Shepherd Dogs

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SUMMARY

The reference values of serum immunoglobulin (Ig) isotype concentrations were defined by a commercial ELISA using sera from individuals of healthy Anatolian Shepherd Dog (Asd). A number of 45 Asd from three different farms (Konya, Sivas and Gemlik) were used in the study. Values of the isotypes were calculated by comparing serum dose-response curves with a reference serum. Mean total serum IgG, IgM, IgA and IgE concentrations were 15.836 ± 0.325 g/L, 1.841 ± 0.086 g/L, 0.951 ± 0.033 g/L and 0.0322 ± 0.0011 g/L, respectively. Regardless of sex and age, dogs from Konya had significantly ($p < 0,05$) higher IgG concentrations than did those from both Sivas and Gemlik. No significant differences were found between the dogs from different farm with respect to serum total IgM, IgA and IgE. The study revealed that to set the standards for a breed it is necessary to consider possible differences between the populations of the breed since IgG concentrations are influenced by environmental factors.

KEY-WORDS : Anatolian Shepherd Dog - immunoglobulin - ELISA - serum - reference value.

RÉSUMÉ

Concentrations sériques totales en IgG, IgM, IgA et IgE chez les bergers d'Anatolie de pure race. Par U.S. UÇAN, V. ALTUNOK et M. MADEN.

Les valeurs de référence des concentrations sériques des 4 isotypes ont été définies par l'utilisation d'immunoglobulines, d'un test ELISA commercial sur les sérums d'individus de la race Berger d'Anatolie. 45 chiens de race Berger d'Anatolie, de trois fermes, ont été utilisés dans l'étude. Les valeurs des isotypes ont été calculées en comparant les courbes de dose-réponse des sérums avec un sérum de référence. La moyenne des concentrations des IgG, IgM, IgA et IgE était respectivement de $15,836 \pm 0,325$ g/L, $1,841 \pm 0,086$ g/L, $0,951 \pm 0,033$ g/L, $0,0322 \pm 0,0011$ g/L. Sans tenir compte du sexe et de l'âge, les chiens de Konya avaient un taux d'IgG significativement ($p < 0,05$) plus haut que ceux de Sivas et Gemlik. Aucune différence significative n'a été trouvée entre les chiens des différentes fermes par rapport aux concentrations sériques des chiens des autres fermes en IgM, IgA et IgE. L'étude a révélé qu'il est nécessaire de considérer les variations entre les populations de l'espèce puisque les concentrations sériques en IgG sont influencées par les facteurs écologiques.

MOTS-CLÉS : chiens Berger d'Anatolie - immunoglobulines - isotypes - ELISA - sérum - valeur de référence.

Introduction

Anatolian Shepherd Dog (Asd) is recognised by International Cynologic Federation with the standard number 331 [4, 8]. Asd is also called as Turkish Shepherd Dog or Kangal Dog in Turkey [13, 14]. Besides their high sensitivity for hearing and smelling, their strength, courage, loyalty and intelligence make them to be exceptionally talented in guarding flocks. Because of these qualities, some individuals were imported in US and England approximately 50 years ago. In the last few decades interest in this dog has been increasing by establishing fan clubs in US, England, Holland, Germany, France and Belgium. Due to their high endurance of extreme variations in heat and cold Asd has

been started to be used as a guard of flocks in Australia, US and some African Countries. Today Asd are bred in US and many European countries especially, England, France, Italy, Switzerland, Germany, Belgium, Holland and Finland [4, 7]. However, there are limited data on the Asd's biological parameters [1, 2].

The purpose of the present study was to gain more insight into some parameters of the immune system in the Asd. Serum concentration differences of the immunoglobulin isotypes will be examined with respect to different sexes, ages and populations. The other reason to study immunoglobulin isotypes is to determine if a relative deficiency in any serum immunoglobulin isotypes in Asd is a breed abnormality.

Materials and methods

At present there are three breeding stocks (Provinces of Sivas, Konya and Gemlik) where breeding programme has been on progress to preserve Asd in Turkey. Pure bred dogs were selected based on criteria given by F.C.I. [8] and by BORG [4]. Blood samples were taken from the farms in these centers. The numbers of blood samples collected from the centers Sivas, Konya and Gemlik were 16, 17 and 12, respectively. Venous blood samples were obtained from three colonies of Asd which were healthy, as determined by physical examination. The serum was stored at - 20°C before assay.

For detection and quantification of total immunoglobulin isotypes (IgG, IgM, IgA, IgE) commercial quantitative ELISA (Sandwich) Immunoassay Kits (E40-118, E40-116, E40-104 and E40-125 ; Bethyl Lab. Inc., Montgomery, TX.) were used. ELISA was conducted according to manufacturer's protocol. However, appropriate serum dilutions for each isotype were required to position the samples in the desired detection range. In a predetermination assay, the samples and antibody/enzyme conjugate (Ab/HRP ; Goat x-Dog IgA HRP) were diluted and assayed to determine working solutions. The working dilution for Ab/HRP was found 1/30.000. The dilutions for the serum samples were 1/50 (in the assays for IgA and IgE) and 1/100 (for IgG and IgM). A ready to use reagent tetramethyl benzidine provided with the kit, was employed as enzyme substrate. The reaction was stopped by adding 2M H₂SO₄ and absorbance read at 450 nm by an ELISA reader (Anthos h+II, Austria). All the samples were run in replicates to provide greater precision.

The study comprised 45 dogs, including 18 sexually intact males and 27 sexually intact females, 0-11 years old. The animals were divided into 4 sub-groups of dogs according to the months of age. The numbers of dogs in the sub-groups of 0-2, 2-4, 4-6, and ≥ 6 months of age were 14, 11, 12, and 8, respectively.

STATISTICS

The Mann-Whitney U-test test was used to compare immunoglobulin concentrations of dogs by sex. Farm- and age-related differences were separately examined for each group by means of Fisher's *F* test (Anova) and Duncan's multiple range analysis. Values of *p* < 0.05 were considered to indicate a significant difference [16].

Results

Mean total serum IgG, IgM, IgA and IgE levels were found 15.836 ± 0.325 g/L, 1.841 ± 0.086 g/L, 0.951 ± 0.033 g/L and 0.0322 ± 0.0011 g/L, respectively in the samples from Asd. The serum immunoglobulin concentrations in three groups of dogs are shown in Table I. No individual showed an absolute deficiency of any isotype. Sex had no statistically significant effect on concentrations of Ig isotypes. Concentrations of IgG, IgM and IgA increased with age. The effects of age on concentrations of IgG, IgM and IgA were statistically significant. However, there was no significant difference between

the four sub-groups of age in respect to IgG. Regardless of sex and age, dogs from Konya had significantly (*p* < 0,05) higher IgG concentrations than dogs from Sivas and Gemlik. Serum concentration of IgG and IgM showed individual variation.

Discussion

These results represent the first report on reference values regarding serum total IgG, IgM, IgE and IgA in the Asd. In literature, reported values for IgG, IgM, IgA and IgE in German Shepherd Dogs 15-17 g/L, 1.3-1.4 g/L, 0.6-0.9 g/L and 0.02-0.03 g/L, respectively [3, 12, 18]. Asd serum IgA and IgE concentrations are found higher than those reported for the German Shepherd Dog and suggested to be primarily influenced by genetic control by this study. GRIOT-WENK et al [12] reported that serum IgA and IgE levels in German Shepherd Dogs seem to also be mainly under genetic control.

The dogs of this study were kept in different environments, which provided an opportunity to evaluate the effects of geographical region as well as sex and age on distribution of serum immunoglobulin isotypes. Perhaps the most surprising data of this study is the differences in IgG concentrations according to the site of dog breeding. The reason for the environment-dependence remains to be elucidated. In an early study [1], it was suggested that the Gemlik population was harbouring very little genetic variations compared to the other two populations. On the other hand, in a study by ALTUNOK et al [2] in which sampling was obtained from the same populations used in this study revealed that the significant differences in the values of inorganic phosphorus, sodium, copper and zinc in different sites indicated that populations within the breed might exhibit different values from each other. ALTUNOK et al [2] then suggested that nutritional and/or other environmental and genetic factors might contribute to these differences. Although the difference between the populations in respect to Ig isotype concentrations except IgG were not significant in this study, Gemlik population showed the lowest concentrations of Ig isotype compared to the others. Therefore, the genetic factors may also contribute to the lowest isotype concentrations. Taken together, it can be suggested that while references for biochemical and immunological parameters for a breed were determined, possible differences between different populations of the breed should be expected. Furthermore, if the populations are selected from different countries or from distant geographic regions, estimated means of the parameters can show more variation.

Increasing serum immunoglobulin concentrations with increasing age has been reported for Beagles [11, 16]. Some increases in serum IgM and IgA concentrations were also found in this study although such increases were not statistically significant.

Low plasma IgA concentrations have been reported to be commonly found in other breeds of clinically healthy dogs [10]. In the dogs examined in this study, systemic IgA levels lower than 0.325 were not observed with the exception of a female individual which showed a concentration of 0.095 g/L IgA.

According to a recent study by GERMAN et al. [9] IgM concentrations in serum are poor markers of secretory Ig status in dogs. However, in people with IgA deficiency, IgA immunocytes in the *lamina propria* of the jejunum have been observed to be replaced by IgM cells [15]. In IgA deficiency, increased concentrations of IgM have been found in secretions, and such IgM has also been reported to be associated with secretory component [5, 6]. However, no significant difference between German Shepherd Dog and other breeds in the concentration of IgG or IgM in the mucosa (tear) has been reported while German Shepherd dogs had significantly lower mucosal IgA concentrations than did the others [9]. On the other hand, BATT et al [3] reported that densities of IgA

producing cells in the small intestine of German Shepherd Dog has not been altered by the local bacterial overgrowth. The authors then suggested that any impairment of mucosal IgA production is more likely to be related to defective synthesis or secretion of IgA than to reduced numbers of IgA-producing immunocytes. Thus, the immunopathogenesis of IgA deficiency in the dog breeds with IgA deficiency seems to be different from those in humans. Since the canine IgA deficiency is defined as an IgA concentration < 0.05 g/L [18], we do not expect any compulsory mechanism for IgA deficiency in Asd, since no IgA-deficient dog has been identified.

		n	IgG (g/L)	IgM (g/L)	IgA (g/L)	IgE (g/L)
Sex	Male	18	15.308 ± 0.597	1.625 ± 0.144	0.929 ± 0.059	0.029 ± 0.0017
	Female	27	16.413 ± 0.475	1.804 ± 0.123	0.921 ± 0.066	0.032 ± 0.0013
	P		0.126	0.281	0.982	0.070
Age (months)	0-2	14	15.777 ± 0.506	1.336 ± 0.108 ^c	0.808 ± 0.094 ^b	0.0319 ± 0.0016 ^{ab}
	2-4	11	16.081 ± 0.761	1.668 ± 1.189 ^{bc}	0.932 ± 0.099 ^b	0.0268 ± 0.0019 ^c
	4-6	12	16.497 ± 0.586	2.036 ± 0.189 ^{ab}	1.006 ± 0.103 ^{ab}	0.0299 ± 0.0015 ^{bc}
	6-	8	17.26 ± 0.842	2.217 ± 0.155 ^a	1.212 ± 0.036 ^a	0.0365 ± 0.0017 ^a
	P		0.472	0.002	0.010	0.004
Site	Sivas	16	15.745 ± 0.565 ^b	1.690 ± 0.151	0.992 ± 0.068	0.0343 ± 0.0014
	Konya	17	16.112 ± 0.583 ^a	2.181 ± 0.098	0.958 ± 0.088	0.0329 ± 0.0017
	Gemlik	12	15.565 ± 0.536 ^b	1.561 ± 0.161	0.887 ± 0.133	0.0323 ± 0.0012
	Total	45	15.836 ± 0.325	1.841 ± 0.086	0.951 ± 0.033	0.0322 ± 0.0011
	P		0.005	0.792	0.752	0.672

^{a-c} Means within columns with no common superscripts are significantly different ($p < 0.05$), according to Duncan's multiple range test. n : Number of the dogs.
P : Significance.

TABLE I. — Mean ± SEM serum immunoglobulin concentrations in the individuals of the Asd.

On the other hand, IgE concentrations were shown to be influenced by age but not by farms suggested that serum IgE levels seemed to be mainly influenced by genetic background. This feature for the serum IgE has also been reported for the German Shepherds and Beagles [12].

In conclusion, reference values for serum total Ig isotypes (IgG, IgM, IgA, and IgE) in healthy Asd were determined for the first time in this study and emphasize the need to consider the influence of environmental factors on different populations of dogs. Moreover, the significance of serum Ig isotype concentrations as diagnostic or pronostic markers has to be examined. Determination of secretory IgA concentrations in healthy Asd and of IgE concentrations in atopic diseases are being investigated. These data are opened up the way to explore the fluctuations of serum Ig isotypes in response to antigenic stimuli or during infectious and/or parasitic diseases in which potential immunomodulation could suppress activity of the immune system.

Acknowledgement

The authors thank Dr S. DERE for assistance with the statistics. Acknowledgement is also gratefully made to Veterinary Faculty of Selçuk University Research Application Unit, Sivas Kangal Farm of The District Government, Sivas Ulas Farm of The District Government, Gemlik Military School of Veterinary Medicine and The Educational Command Centre for giving permission for the collection of the blood samples.

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