Determination of Seropositivity for *Toxoplasma gondii* in Stray Dogs in Istanbul, Turkey

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SUMMARY

The aim of this study was to detect the seropositivity for *Toxoplasma gondii* in stray dogs in Istanbul province. A total of 150 sera from dogs stemming from 5 districts of Istanbul were examined for antibodies against *Toxoplasma gondii* by indirect fluorescence antibody test (IFAT). Anti *T. gondii* antibodies were found in 77 (51.3%) of dogs. Statistically significant differences in seroprevalence were observed between these 5 districts ($P < 0.05$), the Bakirköy and K.burgaz districts being the more infected. No significant association between seropositivity and age or sex was found in the overall population or whatever the district ($P > 0.05$). In conclusion, these results indicate that *T. gondii* infection is fairly common in stray dogs in Istanbul, highlighting the potential threat of toxoplasmosis for humans.

Keywords : *Toxoplasma gondii*, stray dog, IFAT, Istanbul, Turkey

RÉSUMÉ

Séroprévalence de *Toxoplasma gondii* chez les chiens errants, Turquie.

Le but de cette étude est d'évaluer sérologiquement la séroprévalence de l'infestation par *Toxoplasma gondii* chez les chiens errants de la province d'Istanbul. Les anticorps sériques dirigés contre *T. gondii* ont été recherchés par un test d'immunofluorescence indirecte (IFI) sur 150 sérums provenant de chiens de 5 quartiers différents d'lstanbul. Au total, 77 chiens (soit 51.3%) ont été déclarés séropositifs. La séroprévalence a significativement varié selon l'origine des chiens ($p < 0.05$), les quartiers de Bakirköy et de K. burgaz étant les plus infestés. Aucune association significative n'a été mise en évidence entre la séropositivité et 1'âge ou le sexe sur 1'ensemble de la population ou au sein d'un quelconque quartier. En conclusion, ces résultats montrent que la population des chiens errants d'lstanbul est fortement infestée par *T. gondii* et qu'elle constitue un risque potentiel de transmission de la toxoplasmose pour la population humaine.

Mots-clés : *Toxoplasma gondii*, chien errant, IFAT, Istanbul, Turquie.

Introduction

Toxoplasma gondii is a worldwide zoonotic protozoan which can infect virtually all mammals and birds. The definitive hosts of the parasite are the domestic cats and other felines, the sexual cycle of this parasite occurring only in these species [14]. Humans and animals become infected either by consuming food contaminated with sporulated oocysts, or by eating raw meat containing cysts [10, 15]. Dogs have recently been considered as a potential risk factor for *T. gondii* infection in humans due to mechanical transmission of oocysts [20].

The diagnosis of *Toxoplasma* infection is conventionally made by the direct demonstration or isolation of the parasite from biopsy or autopsy material but such techniques are unsuitable for use in large–scale surveys. Therefore immunoserological tests specific for host antibodies have been developed and a variety of tests have been described [4, 28].

Numerous epidemiologic studies of canine toxoplasmosis have been reported in most areas of the world. Prevalence rates in dogs have been varied among countries and diagnostic methods used [25]. Toxoplasmosis in dogs was reported for the first time in Turkey by AKÇAY *et al*. in 1950 [2] and WEILAND and DALCHOW [27] found a seroprevalence of 85% for toxoplasmosis in dogs in Turkey. Data collected from studies carried out in different regions of Turkey in the following years have revealed seropositivity ranged between 11.7% and 85.57% according to the region [5, 7, 12, 18].

Stray dogs constitute a potential risk for public health in big cities, such as Istanbul. Therefore it is important to determine the epidemiological status of toxoplasmosis in this context. In this research, we aimed to determine the prevalence of *T. gondii* in stray dogs in Istanbul.

Materials and methods

STUDY AREA, ANIMALS AND BLOOD COLLECTION

A total of 150 stray dogs (98 males and 52 females) were sampled from various dog rehabilitation centres (DRC) and kennels in five districts of Istanbul and were examined for anti-*Toxoplasma gondii* antibodies (Figure 1). The proportions of males and females sampled in each districts were between 20.7% and 46.7% (overall 34.7%) and between 53.3% and 79.3% (overall 65.3%) respectively. Dogs were 6 months-old to 10 years-old (mean value ± standard deviation:

41.24 ± 20.45 months-old). Three classes of age were defined: 45 dogs were below 2 years-old, 74 dogs were 2 to 5 years-old and 31 dogs were more than 5 years-old. The repartition of dogs according to their age was similar in the 5 districts.

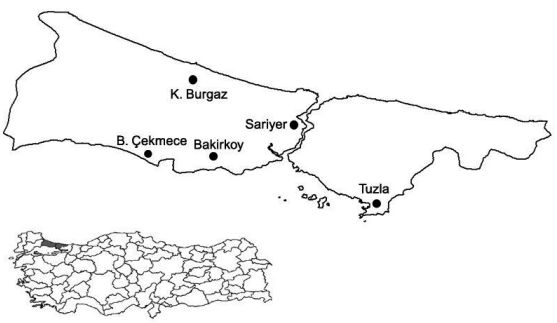

Figure 1. Map of Istanbul province showing the sampled districts.

Istanbul is located on sea level, in north western Turkey. The region has a mild climate. The average annual humidity, temperature and precipitation are 74.9%, 14.6 ºC and 700.4 mm respectively. Blood was drawn from the cephalic vein of each dog and after clotting at room temperature, serum was obtained by centrifugation at 1 000 g for ten minutes at room temperature and stored at –20 °C until used.

SEROLOGICAL TESTING

Preparation of *T. gondii* antigens :

T. gondii RH strain which were used as antigen for IFAT were obtained by 48 hours intraperitoneal passages from 3-5 weeks old white mice (*Mus musculus variety albino*). The animals were killed 48 hours after injection. After infection tachyzoites were collected by repeated flushing of the peritoneal cavity with PBS (pH 7.4) by centrifugation at 1000 g. Viability of the tachyzoites was evaluated by trypan blue exclusion test using a Neubauer chamber. They are considered as ready to use after quality control for IFAT detection of toxoplasmosis which could detect both IgG and IgM from human and animal sera.

Principle of IFAT :

The IFAT was performed using standard procedures at the Parasitology Laboratory of the Veterinary Control and Research Institute, Konya. Briefly, *Toxoplasma* tachyzoites were washed by centrifugation and then resuspended at a concentration of 2 _10^6 ml^{-1} in saline buffer. Sera samples were diluted from 1:16 until 1:4096 in phosphate buffered saline (PBS 0.015 M, pH 7.2). Ten micro litres of each diluted sera were pipetted in each delimited circle on the slides previously adsorbed with *T. gondii* antigen from tachyzoites. The slides were incubated at 37°C for 30 minutes in a wet chamber. Then they were washed three times in PBS, dried and were incubated for 30 minutes, at 37°C with a FITC conjugated anti-dog IgG antibody (Sigma F- 4012) diluted 1: 32 in PBS.

The slides were washed and air dried. A drop of glycerol buffer was added and each slide was covered with a cove slip. Finally the samples were observed under the immunofluorescent microscope (Olympus Mod BH2, Tokyo, Japan). Titres of 1:64 and above were accepted as positive [22-24]. Positive and negative control sera were obtained from dogs previously tested by conventional serological assays (SFDT, IFAT).

STATISTICAL ANALYSIS

Results were statistically analysed using the chi-square test by analytical software package (Statistix version 1.0., 1996). Differences were considered as significant when p values were less than 0.05.

Results

From the total of 150 dogs, 77 (51.3%) were seropositive for anti-*T. gondii* antibodies. Antibodies against *T. gondii* were found in all 5 districts sampled. Dogs sampled in Bakirköy had the highest prevalence (64%) of toxoplasmosis while the lowest prevalence was found among dogs from Sarıyer (31.6%) as shown in Table I ($p < 0.05$). The seropositivity of toxoplasmosis in dogs from Kemerburgaz, Tuzla and Buyukçekmece was 60.7%, 55.2% and 53.3% respectively. The distribution of the anti *Toxoplasma* antibody titres showed similar profiles in the overall positive dogs and in positive animals from each district (Figure 2). Among positive dogs, low titres (1:64 and 1:128) were essentially detected (84% in the overall population of stray dogs / from 44% to 75% according to the district) whereas sera with highest titres (1:1024, 1:2048 and 1:4096) were rare (7% in the overall population / between 5.8% and 9% according to the district) (Figure 2)

A highest prevalence (67.7%) of anti *Toxoplasma* antibodies was detected in relatively old dogs (age > 5 years) whereas the lowest value (44.5%) was obtained in adult dogs (age ranged from 2 to 5 years) (Table 2). Again, low titres (1:64 and 1:128) were the most frequently encountered in all classes of age (Figure 3). Higher titres (1:1024 to 1:4096) were only observed in adult and old dogs. Nevertheless, no significant difference was evidenced between the different age classes of dogs in the overall sampled population or in the dog population of a given district.

The *T. gondii* seroprevalence was 0.500 and 0.538 in females and males respectively (Table 3). The distribution of the anti *Toxoplasma* antibody titres was similar in both sexes with roughly the same proportions of low litres (1:64 and 1:128 in 82% of males and in 86% of females). Moreover, the proportions of infested males did not significantly differ from the proportions of infested females in the overall dog population or in each considered district.

Consequently, no significant association was found between *T. gondii* seropositivity and age or sex ($p > 0.05$).

Districts	Number of sampled dogs	Positive responses	
		Number	Percentages
Sariyer	38	12	31.6[a]
B. Cekmece	30	16	53.3[ab]
Tuzla	29	16	55.2[ab]
K. Burgaz	28	17	60.7[b]
Bakirköy	25	16	64.0[b]
Total	**150**	**77**	**51.3**

Different superscripts in the same column indicate significant differences ($p < 0.05$).

TABLE 1. Seroprevalence of *T. gondii* in stray dogs according to the sampled district in Istanbul, Turkey.

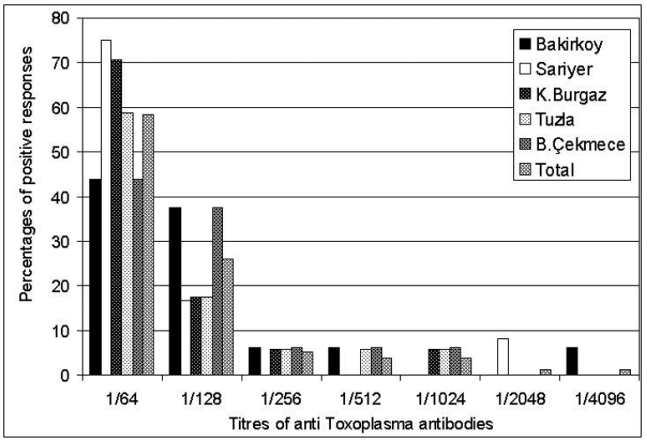

Figure 2. Distribution of the titres of anti *Toxoplasma* antibodies in the overall population of stray dogs according to the district of Istanbul, Turkey.

Population of stray dogs		Age classes (in years)		
		[0 - 2[	[2 - 5]	[5 and more]
Overall population				
	Number of dogs	45	74	31
	Number of positive dogs	23	33	21
	Theoritical values	23	38	16
	Observed seroprevalence	0.511	0.445	0.677
District population				
***Sariyer*:**	Number of dogs	16	18	4
	Number of positive dogs	5	6	1
	Theoritical values	5	6	1
	Observed seroprevalence	0.312	0.333	0.250
***B. Cekmece*:**	Number of dogs	4	24	2
	Number of positive dogs	3	11	2
	Theoritical values	2	13	1
	Observed seroprevalence	0.750	0.458	1.000
***Tuzla*:**	Number of dogs	12	12	5
	Number of positive dogs	6	6	4
	Theoritical values	7	7	3
	Observed seroprevalence	0.500	0.500	0.800
***B. Burgaz*:**	Number of dogs	5	12	11
	Number of positive dogs	4	7	6
	Theoritical values	3	7	7
	Observed seroprevalence	0.800	0.583	0.545
***Bakirköy*:**	Number of dogs	8	8	9
	Number of positive dogs	5	3	8
	Theoritical values	5	5	6
	Observed seroprevalence	0.625	0.375	0.888

TABLE 2. Seroprevalence of *T. gondii* in stray dogs from Istanbul, Turkey for the overall population and for dog population of each district according to the age distribution.

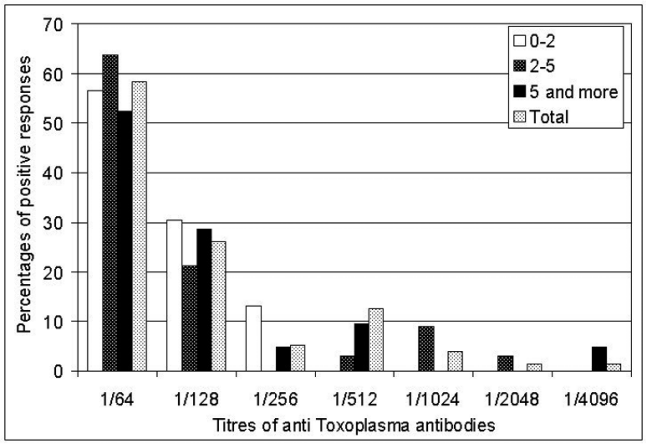

Figure 3. Distribution of the titres of anti *Toxoplasma* antibodies in the overall population of stray dogs from Istanbul, Turkey according to the age (in years) distribution.

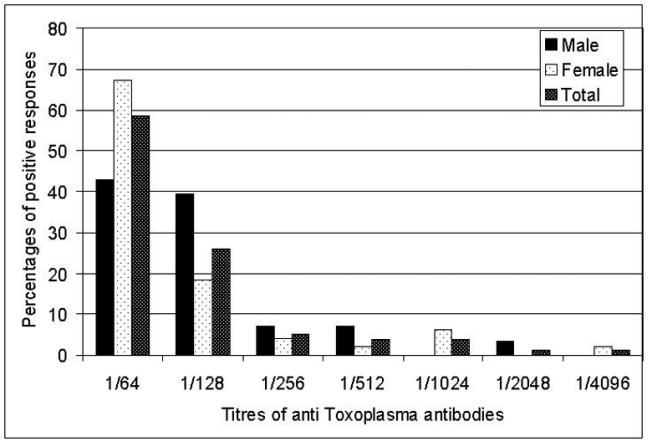

Figure 4. Distribution of the titres of anti *Toxoplasma* antibodies in the overall population of stray dogs from Istanbul, Turkey according to the sex.

Population of stray dogs		Males	Females
Overall population			
	Number of dogs	52	98
	Number of positive dogs	28	49
	Theoritical values	27	50
	Observed seroprevalence	0.538	0.500
District population			
***Sariyer*:**	Number of dogs	12	26
	Number of positive dogs	4	8
	Theoritical values	4	8
	Observed seroprevalence	0.333	0.307
***B. Cekmece*:**	Number of dogs	14	16
	Number of positive dogs	8	8
	Theoritical values	7	8
	Observed seroprevalence	0.571	0.500
***Tuzla*:**	Number of dogs	6	23
	Number of positive dogs	5	11
	Theoritical values	3	13
	Observed seroprevalence	0.833	0.478
***B. Burgaz*:**	Number of dogs	11	17
	Number of positive dogs	6	11
	Theoritical values	7	10
	Observed seroprevalence	0.545	0.647
***Bakirköy*:**	Number of dogs	9	16
	Number of positive dogs	5	11
	Theoritical values	6	10
	Observed seroprevalence	0.555	0.688

TABLE 3. Seroprevalence of *T. gondii* in stray dogs from Istanbul, Turkey for the overall population and for dog population of each district according to the sex.

Discussion

The *T. gondii* infection in dogs is world widely distributed, with different prevalence rates ranging from 0% to 100% in the different countries [6, 8, 9, 21, 25, 26]. The prevalence of 51.3 % found in this study using the indirect immunofluores cent antibody test is lower than reported for dogs in Rondonia, Brazil (76.4%) [8] but is higher than reported in Austria (26%) [26] and in Paraiba, Brazil (45.1%) [6] with the same or equivalent test. In the present study, the seroprevalence found in stray dogs in Istanbul was 51.3%, and this result is an agreement with previous studies conducted on dogs in different parts of Turkey [5, 7, 12, 18] that reported values comprised between 11.75 and 85.57%. It is therefore inaccurate to compare prevalence data of studies which used different serological tests, sample sizes and period of surveys, types of dog population and cut-off values [6, 9, 23, 24].

Serum antibodies to *T. gondii* were found in 77 out of the 150 dogs sampled. The prevalence of Toxoplasma infection is variable according to the districts. The highest seroprevalence was observed in Bakirköy (64%) and K.burgaz (60.7%), while the lowest seropositivity was found in Sariyer (31.6%). The statistically significant differences observed could be due to the climatic variations from one district to another and to the frequency of cats in the farms, which are essential elements in epidemiological studies. Higher prevalence found in the K.burgaz and Bakirköy indicate that the dogs found in two districts were exposed to an environment contaminated with more *T. gondii* oocysts, compared to those found in the other districts. A positive association was observed between the seropositivity for *T. gondii* and the presence of cats in the household, indicating that the presence of cats and the close contact with this specie is important in the epidemiology of the disease [11]. Infected cats excrete Toxoplasma oocysts, which after sporulation become infectious to man and animals and remain infectious for a long time [16]. This finding indicates that further researches should be performed with regard to *T. gondii* infections in cats from Istanbul. Higher prevalence rates of toxoplasmosis in warm and moist areas compared to cold and dry ones is attributed to the lengthening of *T. gondii* oocysts viability in moist or in humid environments [13]. Istanbul is a warm and moist area that helps *T. gondii* oocysts to maintain their viability.

For many authors [3, 8, 19], age is an important factor: older pets were more frequently infected by *T. gondii* than younger pets. But this fact is controversial. Indeed AHMED *et al.* [1] found that the highest frequency of infection occurred in younger dogs up to four years of age and the lowest in older dogs. HUCHINSON and JACKSON [17] reported also that toxoplasmosis susceptibility could vary according to the age and that younger or older animals would be the more affected. However our results indicated that there was no significant difference between age groups ($P > 0.05$). In the same way, no significant association was found between *T. gondii* infection and sex in stray dogs from Istanbul, in agreement with other studies [3, 8, 19].

From the results presented here, it appears that *T. gondii* infection is highly prevalent in stray dogs in Istanbul. Toxoplasma infection among animals is of great importance, because some of the infected animals play a notable role as the source for human infection. Finally we emphasize that further studies are needed to clarify the impact of toxoplasmosis on the animal industry and losses due to clinical toxoplasmosis in livestock in Turkey.

References

1.– AHMED A.A., GAAFAR S.M., WEIRICH W.E., KAMITZ C.L.: Relationship of toxoplasma infections to other diseases in dogs. *Vet. Parasitol*., 1983, **12**, 199-203.

2.– AKÇAY _., PAMUKÇU M., BARAN S.: Bir köpekte ilk Toxoplasmose observasiyonu. *Türk. Vet. Hek. Derg*., 1950, **20**, 245-254.

3.– ALI C.N., HARIS J.A., WATKINS J.D, ADESIYUN A.A.: Seroepidemiology of *Toxoplasma gondii* in dogs in Trinidad and Tobago. *Vet. Parasitol*., 2003, **113**, 179-187.

4.– ANDERSON S.E. and REMINGTON J.S.: The diagnosis of toxoplasmosis. *South Med. J*.,1975, **68**, 1433-1443.

5.– ASLANTAS O., OZDEMIR V., KILIÇ S., BABUR C.: Seroepidemiology of leptospirosis, toxoplasmosis and leishmaniosis among dogs in Ankara, Turkey. *Vet. Parasitol*.**,** 2005, **129**, 187-191.

6.– AZEVEDO S.S., BATISTA C.S.A., VASCONCLLOS S.A., AGUIAR D.M., RAGOZO A.M.A., RODRIGUES A.A.R., ALVES C.J., GENNARI S.M: Seroepidemiology of *Toxoplasma gondii* and *Neospora caninum* in dogs from the state of Paraiba, Northest region of Brazil. *Res. Vet . Sci*., 2005, **79**, 51-56.

7.– BABUR, C., BIYIKOGLU, G., PISKIN, F.Ç., ERDAL, N.: Seroprevalence of toxoplasmosis on stray dogs in Istanbul. *Acta Parasitol. Turcica*., 1997, **21**, 413-416.

8.– CÂNON-FRANCO W.A., BERGAMASCHI D.P., LABRUNA M.B., CAMARGO L.M.A., SILVA J.C.R., PINTER A., GENNARI S.M.: Occurence of anti-*Toxoplasma gondii* antibodies in dogs in the Urban area of Monte Negro, Rondonia, Brazil. *Vet. Res. Com*., 2004, **28**, 113-118.

9.– DUBEY J.P.: Toxoplasmosis in Am. dogs. *Canine Prac*., 1985, **12**, 7-28.

10.– DUBEY J.P: Toxoplasmosis. *J. Vet. Med. Assoc*., 1994, **205**, 1593-1598.

11.– DUBEY, J.P and BEATTIE, C.P. (Eds): Toxoplasmosis of animals and man. CRC Pres, Boca Raton, Florida, 1988, 1-220.

12.– EREN H., SARI C., TURGAY N., ERTUG S.: Screening of anti-Toxoplasma IgG antibodies using IFA in healthy petdogs in Ayd_n. Acta *Parasitol. Turcica*., 2002, **26**, 352-354.

13.– FAYER, R.: Toxoplasmosis update and public health implications. *Can. Vet. J*., 1981, **22**, 344-352.

14.– FRANKEL, J.K., DUBEY, J.P., MILLER, N.L.: *Toxoplasma gondii* in cats: Fecal stages identified as coccidian oocysts. *Science*., 1970, **167**, 893-896.

15.– FRANKEL, J.K.: Physiopathology of toxoplasmosis. *Parasitol. Today*.,1988, **4**, 273-278.

16.– GHORBANI M., HAFIZI, A., SHEGERFCAR M.T., REZAIAN M., NADIM A., ANWAR M., AFSHAR, A.: Animal toxoplasmosis in Iran. *J. Trop. Med. Hyg*.,1983, **86**, 73-76.

17.– HUCHINSON W.M and JACKSON M.H.: The prevalence and source of Toxoplasma infection in the environment. *Adv. Parasitol*., 1989, **28**, 55-86.

18.– INCI A., BABUR C., ÇAM Y., IÇA A.: The seroprevalence of *Toxoplasma gondii* (Nicolle ve Manceaux, 1908) in dogs around Kayseri. *Acta Parasitol. Turcica*., 2002, **26**, 221-223.

19.– LIN D.S.: Seroprevalences to *Toxoplasma gondii* in privately-owned dogs in Taiwan. *Prev. Vet. Med*., 1998, **35**, 21-27.

20.– LINDSAY D.S., DUBEY J.P., BUTLER J.M., BLAGBURN B.L.: Mechanical transmission of *Toxoplasma gondii* oocysts by dogs. *Vet. Parasitol*., 1997, **73**, 27-33.

21.– MINEO T.W.P., SILVA D.A.O., COSTA G.H.N., VON ANCKEN A.C.B., KASPER L.H., SOUZA M.A., CABRAL D.D., COSTA A.J., MINEO J.R.: Detection of IgG antibodies to *Neospora caninum* and *Toxoplasma gondii* in dogs examined in a veterinary hospital from Brazil. *Vet. Parasitol*., 2001, **98**, 239-245.

22.– OZCEL, M.A.: Immunofluoresans ve parasitolojide uygulanmas_. Ege Üniversitesi T_p Fak. Yay. No: 108, Bornova, _zmir. 1978.

23.– SILVA D.A.O., CABRAL D.D., BERNARDINA B.L.D., SOUZA M.A., MINEO J.R.: Detection of *Toxoplasma gondii* specific antibodies in dogs. A comparatire study of immunoenzymatic, immunofluorescent and haemagglutination titres. *Mem. Inst. Oswaldo Cruz*., 1997, **92**, 785-789.

24.– SILVA N.M., LOURENÇO E.V., SILVA D.A.O., MINEO J.R.: Optimisation of **c**ut-off titres in *Toxoplasma gondii* specific ELISA and IFAT in dog sera using immunoreactivity to SAG-1 antigen as a molecular marker of infection. *Vet. J.,* 2002, **163**, 94-98.

25.– TENTER A.M, HECKEROTH A.R, WEISS L.M.: *Toxoplasma gondii*: from animals to humans. *Int. J. Parasitol*., 2000, **30**, 1271-1258.

26.– WANHA, K., EDELHOFER, R., GABLER-EDUARDO, C., PROSL, H.: Prevalence of antibodies against *Neospora caninum* and *Toxoplasma gondii* in dogs and foxes in Austria. *Vet. Parasitol*., 2005, **128**, 189-193.

27.– WEILAND, V.G and DALCOW, W.: Toxoplasma infektionen bei Haustieren in der Türkei (Serologische untersuchungen im Sabin-Feldman test). *Berl. Münch. Tierarztl. Wschr*., 1970, **85**, 65-68.

28.– WILSON, M., WARE, D.A., JURANEK, D.D.: Serologic aspects of toxoplasmosis. *J. Am. Vet. Med. Assoc*., 1990, **196**, 277-280.