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Detection of Anti-*Neospora caninum* Antibodies in Cattle in Adana Province of Turkey

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ABSTRACT *Neospora caninum* is an important aborting agent in cattle. In addition to aborts, foetal deaths, resorption, mummification, autolysis, stillbirth, symptomatic or persistently infected calf births, decreased milk production, repeat breeder and premature culling occur in infected herds, which leads to economic losses. The aim of this study was to determine the prevalence of anti-*N. caninum* antibodies in cattle in Adana province of Turkey. For this purpose, 225 blood samples were collected from cattle in different breed, age and sex in 15 counties of Adana. Obtained sera were examined with c-ELISA test. At the end of the study, anti-*N. caninum* antibodies were detected in 24 out of 225 animals (10.7%). While the counties were grouped based on their altitudes (0-99 m, 100-500 m and ≥501 m), the cattle were grouped in respect of breeds (Holstein, crossbreed, others), ages (≤4 and ≥5) and sex (male and female). Correlation between seropositivity and variants (altitude, breed, age and sex) was investigated with chi-square (X^2) test. There was no statistical correlation between seropositivity and altitude, breed and sex ($P>0.05$). Seropositivity rates were 15.8% at age of ≥5 and 6.9% at age of ≤4 in cattle. The correlation between age and seropositivity was statistically significant ($P<0.05$). In this study, we evaluate the exposure rates of cattle to *N. caninum* as well as the correlation between seropositivity and different variants in Adana province of Turkey.

Keywords: *Neospora caninum, Cattle, c-ELISA, Adana, Turkey*

ÖZ

Türkiye'nin Adana Yöresi Sığırlarında Anti-*Neospora caninum* Antikorlarının Araştırılması

Neospora caninum sığırların önemli protozoal abort etkenlerinden biridir. Sığırlarda Neospora enfeksiyonlarında abortlara ilave olarak fötal ölüm ve rezorbsiyon, mumifikasyon, otoliz, ölü doğum, canlı ancak klinik semptom gösteren ya da persiste enfekte yavru doğumları şekillenir. Enfeksiyonun görüldüğü sürülerde ayrıca süt veriminde düşme, döl tutamama ve erken damızlıktan ayırma gibi ekonomik kayıplar da ortaya çıkabilir. Bu çalışmanın amacı Adana yöresi sığırlarında anti-*N. caninum* antikorlarının yaygınlığını belirlemekti. Bu amaçla Adana'nın 15 ilçesinden farklı ırk, yaş ve cinsiyette toplam 225 sığırdan kan alındı ve elde edilen serumlar c-ELISA testi ile incelendi. Çalışma sonucunda prevalans %10.7 (24/225) olarak belirlendi. İlçeler rakım özelliklerine göre 0-99 m, 100-500 m ve ≥501 m olarak, sığırlar ırk özelliklerine göre Holstein, melez ve diğerleri, yaşlarına göre de ≤4 ve ≥5 olarak gruplandırıldı. Rakım, ırk, yaş ve cinsiyet değişkenleri ile seropozitiflik arasındaki ilişki ki-kare (X^2) testi ile analiz edildi. Rakım, ırk ve cinsiyet değişkenleri ile seropozitiflik arasında istatistiki açıdan bir fark bulunamadı ($P>0.05$). 5 yaş ve üstü sığırlarda seropozitiflik oranı %15.8 iken, bu oranın 4 yaş ve altındaki sığırlarda %6.9'a düştüğü ve bu değerlerin istatistiki açıdan önemli olduğu ($P<0.05$) tespit edildi. Bu çalışma ile Adana yöresi sığırlarının etkene maruziyet durumları ve farklı değişkenler ile hastalığın yaygınlığı arasındaki ilişki değerlendirilmiştir.

Anahtar Kelimeler: *Neospora caninum, Sığır, c-ELİSA, Adana, Türkiye*

INTRODUCTION

Abort in cattle is described as the loss of foetus between 42 and 260 days of gestation (Al-Samarai et al. 2012). Mechanic-traumatic factors, nutritional deficiencies, hormonal disorders and infections (bacteria, viruses and parasites) play important role in abortion etiology (Tulu et al. 2018). *Neospora caninum*, an obligate intracellular protozoon in the phylum of Apicomplexa, is one of the most important infectious agents in cattle abortion (Schares et al. 1998; Anderson et al. 2000; Haddad et al.2005). In the domestic cycle of the disease, cattle, sheep, goat, buffalo and horses are intermediate hosts, while dogs are final hosts of *N.caninum* (Dubey 2003; Dubey and Schares 2011).

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The disease is transmitted horizontally and vertically between animals (Dubey 2007). Cattle are infected by contaminated food and water with sporulated oocysts in horizontal transmission, and carry the infection to their offsprings by endogenous and exogenous transplacental ways (Anderson et al. 2000). Endogenous transplacental transmission causes persistently infected individuals and expansion of the disease in cattle herds, while exogenous transplacental transmission causes abortion storms (Anderson et al. 2000; Dubey and Schares 2006).

In addition to aborts, foetal deaths, resorption, mummification, autolysis, stillbirth, symptomatic or persistently infected calf births, decreased milk production, repeat breeder and premature culling may occur in infected herds, which causes economic losses (Dubey 2003; Simsek et al. 2008; Piskin and Utuk 2009).

While the disease is a suspected cause of abortion in cattle herds, the final diagnosis is done through the examination of aborted foetus with histopathologic and molecular techniques (PCR, DNA sequencing). The prevalence of the disease in final and intermediate hosts can also be determined through serologic techniques (IFAT, ELISA) (Schares et al. 1999; Dubey 2003; Haddad et al. 2005; Guido et al. 2016).

Our hypothesis was that this study gives information about the seroprevalence of *N. caninum* in the sixth biggest and specific province of Turkey and one of the most detailed study about Neosporosis in Turkey and also reviewed the studies about Neosporosis in Turkey and worldwide.

The aim of this study was to determine the prevalence of anti-*N. caninum* antibodies as well as the correlation between seropositivity and different variants (altitude, breed, age and sex) in cattle in Adana, the sixth largest province of Turkey.

MATERIALS and METHODS

Adana is located to the south of the Taurus Mountains and on both sides of the Seyhan River. The city has major transportation routes connecting Europe to Asia. The altitude in Adana changes between 10 to 1453 m from one county to another. In this province, the Mediterranean climate is found in the lowlands, while the mountainous areas have a continental climate.

A total of 225 blood samples were collected from cattle in different breed, age and sex from 15 counties of Adana. Obtained sera were stored at -20°C until used. While the counties were grouped based on their altitudes (0-99 m, 100-500 m and ≥501 m) (Figure 1) the cattle was grouped in respect of breeds (Holstein, cross-breed, others), ages (≤4 and ≥5) and sex (male and female) (Table 1) Correlation between seropositivity and variants (altitude, breed, age and sex) were investigated with chi-square (X^2) test.

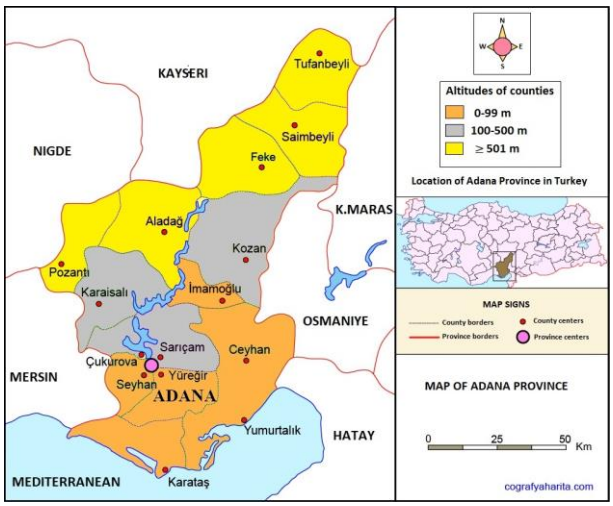

Figure 1. Location of Adana**,** study areas and their altitudes (Anonymous 2017).

Anti-*N.caninum* antibodies were detected with commercially available c-ELISA test kit (VMRD, USA) according to the manufacturer's recommendation. Percent inhibition values with ≥30 were accepted as positive while <30 accepted as negative.

This study was approved by the Ethics Committee of Adana Veterinary Control Institute (20.09.2016/2833).

RESULTS

At the end of the study, anti-*N.caninum* antibodies were detected in 24 out of 225 animals (10.7%). There was no statistical correlation between seropositivity and altitude, breed and sex (P>0.05). Seropositivity rates were 15.8% at age of ≥5 and 6.9% at age of ≤4 in cattle. The correlation between age and seropositivity was statistically significant (P<0.05) (Table 1).

Table 1. Number of cattle, seropositivity rates and correlation among different variants

Epidemiologic Data		No. Tested (n)	Seropositivity n (%)	*P*
Altitude (m)	0-99	79	9 (11.4)	0.858
	100-500	69	8 (11.6)	
	≥501	77	7 (9.1)	
Breed	Holstein	170	20 (11.8)	0.576
	Cross-Breed	34	3 (8.8)	
	Other	21	1 (4.8)	
Age	≤4	130	9 (6.9)	0.033
	≥5	95	15 (15.8)	
Sex	Female	206	22 (10.7)	0.213
	Male	19	2 (10.5)	

Table 2. Serologic studies about *Neospora caninum* in cattle from different parts of the world

Continent	Country	Test	No. tested	No. positive	% positive	Reference	Mean Prevalence (%)	World (%)
Africa	Nigeria	ELISA	174	6	3.4	(Ayinmode et al. 2017)		
	Morocco	iELISA	176	15	8.52	(Lucchese et al. 2016)		
	Egypt	iELISA	301	57	18.9	(Fereig et al. 2016)	**14.78 (598/4046)**	
	Ethiopia	cELISA	2334	335	13.3	(Asmare et al. 2013)		
	Algerian	IFAT	799	157	19.64	(Ghalmi et al. 2012)		
	Sudan	cELISA	262	28	10.7	(Ibrahim et al. 2012)		
Asia	Iran	ELISA	1500	395	26.33	(Hosseininejad et al. 2017)		
	Iran	ELISA	492	63	12.80	(Gharekhani et al. 2014)		
	Iran	ELISA	266	28	10.5	(Nematollahi et al. 2011)		
	Iran	ELISA	237	76	32	(Youssefi et al. 2009)		
	Iran	ELISA	285	36	12.6	(Fard et al. 2008)		
	China	iELISA	510	210	41.2	(Qian et al. 2017)		
	China	ELISA	370	70	18.9	(Xia et al. 2011)		
	China	ELISA	540	72	13.3	(Wang et al. 2010)		
	Indonesia	ELISA	991	165	16.6	(Ichikawa-Seki et al. 2016)	**16.05 (1732/10787)**	
	Thailand	cELISA	445	52	11.7	(Jittapalapong et al. 2008)		
	Thailand	cELISA	549	30	5.5	(Kyaw et al. 2004)		
	Thailand	IFAT	904	54	6	(Suteeraparp et al. 1999)		
	Japan	IFAT	2420	139	5.7	(Koiwai et al. 2006)		
	Japan	IFAT	Dairy 145	29	20	(Koiwai et al. 2005)		
	Japan	IFAT	Beef 65	1	1.5	(Koiwai et al. 2005)		
	Pakistan	cELISA	641	277	43	(Nazir et al. 2013)		
	India	cELISA	427	35	8.2	(Meenakshi et al. 2007)		
Europe	Slovak	iELISA	490	118	24.1	(Špilovská et al. 2015)		
	Czech Republic	cELISA	546	3	0.5	(Bártová et al. 2015)		
	Czech Republic	ELISA/IFAT	407	13	3.19	(Václavek et al. 2003)		
	Serbia	cELISA	356	54	15.4	(Kuruca et al. 2013)		
	Western Romania	cELISA	376	104	27.7	(Imre et al. 2012)		
	Southern Romania	iELISA	258	104	40.31	(Mitrea et al. 2012)		
	Romania	ELISA	901	312	34.6	(Gavrea et al. 2011)		
	Italy	ELISA	3749	1114	29.7	(Gennero et al. 2007)		**22.88 (11224/49058)**
	Italy	ELISA	1140	126	11	(Otranto et al. 2003)	**23.69 (2681/11318)**	
	France	ELISA	1924	107	5.6	(Ould-Amrouche et al. 1999)		
	Germany	ELISA	Beef herd 106	43	41	(Bartels et al. 2006)		
	Germany	ELISA	Dairy herd 100	50	50	(Bartels et al. 2006)		
	Netherlands	ELISA	Beef herd 82	58	71	(Bartels et al. 2006)		
	Netherlands	ELISA	Dairy herd 108	86	80	(Bartels et al. 2006)		
	Spain	ELISA	Beef herd 372	171	46	(Bartels et al. 2006)		
	Spain	ELISA	Dairy herd 291	184	63	(Bartels et al. 2006)		
	Sweden	ELISA	Dairy herd 112	34	30	(Bartels et al. 2006)		
South America	Brazil	IFAT	75	5	6.7	(Cerqueira-Cézar et al. 2017)		
	Brazil	IFAT	575	559	97.2	(Cerqueira-Cézar et al. 2017)		
	Bahia	IFAT	447	63	14	(Gondim et al. 1999)		
	Goias	IFAT	456	135	29.6	(Melo et al. 2006)		
	MatoGrosso do Sul	IFAT	2448	449	14.9	(Oshiro et al. 2007)		
	Minas Gerais	IFAT	575	559	97.2	(Guedes et al. 2008)		
	Minas Gerais	IFAT	1204	260	21.6	(Bruhn et al. 2013)		
	Para	IFAT	500	260	52	(Silva et al. 2017)		
	Parana	ELISA	1263	423	33	(Locatelli-Dittrich et al. 2008)	**53.76 (4797/18922)**	
	Pernambuco	IFAT	469	163	31.7	(Silva et al. 2008)		
	Rio Grande do Sul	IFAT	1549	276	17.8	(Corbellini et al. 2006)		
	Rondonia	IFAT	1011	114	11.2	(Aguiar et al. 2006)		
	SantaCatarina	IFAT	1518	466	30.6	(Fávero et al. 2017)		
	Sao Paulo	IFAT	1027	107	10.4	(Cardosa et al. 2012)		
	Tocantins	IFAT	192	48	25.0	(Martins at al. 2011)		
	Argentina	IFAT	290	59	20.3	(Moore et al. 2003)		
	Paraguay	ELISA	879	262	29.8	(Osawa et al. 2002)		
	Uruguay	ELISA	4444	589	13.25	(Bañales et al. 2006)		
North America	Mexico	ELISA	596	69	11.6	(García-Vázquezet et al. 2009)		
	Mexico	ELISA	187	110	59	(García-Vázquezet et al. 2002)	**35.53 (1416/3985)**	
	Costa Rica	iELISA	2743	1185	43.3	(Romero et al. 2005)		
	Venezuela	ELISA	459	52	11.3	(Lista-Alves et al. 2006)		

Table 3. Serologic studies about *Neospora caninum* in cattle from different parts of Turkey

Region	Province	Test	No. tested	No. positive	% positive	Reference	Mean Prevalence (%)	World (%)
Mediterranean	Burdur	cELISA	400	21	5.3	(Adanir et al. 2015)	**5.3 (21/400)**	
Marmara	Sakarya	cELISA	92	10	9.2	(Öncel et al. 2003)	**11.6 (52/470)**	
	Tekirdağ	ELISA	124	7	5.6	(Bıyıkoğlu et al. 2003)		
	Kırklareli	ELISA	150	15	10	(Bıyıkoğlu et al. 2003)		
	Bursa	cELISA	104	20	19.23	(Eşki et al. 2016)		
Aegean	Afyonkarahisar	cELISA	485	102	21.03	(Çelik et al. 2013)	**21,03 (102/485)**	
Central Anatolia	Kayseri	cELISA	186	13	7	(İça et al. 2006)		
	Ankara	ELISA	453	46	10.15	(Vural et al. 2006)		
	Eskişehir	ELISA	387	21	5.43	(Vural et al. 2006)		
	Çankırı	ELISA	418	29	6.93	(Vural et al. 2006)		
	Kırıkkale	ELISA	434	142	32.72	(Vural et al. 2006)		
	Kırşehir	ELISA	409	80	19.55	(Vural et al. 2006)	**15.89 (654/4114)**	
	Yozgat	ELISA	369	75	20.32	(Vural et al. 2006)		
	Nevşehir	ELISA	392	20	5.10	(Vural et al. 2006)		**13.06 (1023/7830)**
	Kayseri	ELISA	425	46	10.82	(Vural et al. 2006)		
	Aksaray	ELISA	261	91	34.9	(Öcal et al. 2014)		
	Niğde	cELISA	264	70	26.51	(Karatepe and Karatepe 2016)		
	Kırşehir	cELISA	116	21	18.1	(Yıldız et al. 2017)		
Eastern Anatolia	Bingöl	cELISA	149	7	4.69	(Aktaş et al. 2005)	**6.55 (120/1832)**	
	Elazığ	cELISA	120	18	15	(Aktaş et al. 2005)		
	Malatya	cELISA	100	4	4	(Aktaş et al. 2005)		
	Muş	cELISA	144	7	4.86	(Aktaş et al. 2005)		
	Kars	ELISA	301	6	2	(Akca et al. 2005)		
	Elazığ	cELISA	183	15	8.19	(Şimşek et al. 2008)		
	Van	cELISA	450	22	4.88	(Alan et al. 2011)		
	Erzurum	cELISA	385	41	10.65	(Balkaya et al. 2012)		
Southeastern Anatolia	Şanlıurfa	cELISA	305	23	7.5	(Sevgili et al. 2005)	**7.5 (23/305)**	
Black Sea	Samsun	cELISA	224	51	22.7	(Kaya S et al. 2011)	**22.7 (51/24)**	

DISCUSSION and CONCLUSION

Different studies have been conducted to evaluate the correlation between the seroprevalence of *N.caninum* and several variables such as breed, age, sex, altitude, etc., but have yielded different results. In some studies, a statistically significant correlation is found between the seroprevalence of *N.caninum* and the variants like breed, age, sex and climatic conditions (Guimarães et al. 2004; Jittapalapong et al. 2008; Asmare et al. 2013; Nazir et al. 2013; Macedo et al. 2017; Yıldız et al. 2017), while others indicate the opposite (Aktaş et al. 2005; Simsek et al. 2008; Ibrahim et al. 2012; Imre et al. 2012; Fereig et al. 2016; Gharekhani et al. 2014; Adanir et al. 2015). In this study, no correlation was found between the seropositivity and the variants like breed, sex, and altitude (P>0.05). However, the rate of seropositivity in cattle aged ≥5 was 15.8%, while it dropped to 6.9% in cattle aged ≤4, and these values were found to be statistically significant (P<0.05). The reason for this is considered to be the host-parasite interaction in cattle, increasing with age.

When the seroprevalence values in different studies from around the world are considered, it can be observed that there exists a dramatic fluctuation in respect of the serological methods used, the differences in cut-off values even when the same method is employed, sampling methods as well as sample size (Wapenaar et al. 2007; Guido et al. 2016; Cerqueira-Cézar et al. 2017).

For instance, in a study conducted in 2003 in the Minas Gerais region of Brazil (Ragozo et al. 2003), the rate of positivity was found to be 11.1% (4/36) at 1/25 titer by IFA test, while another study in 2008 reported the rate of positivity in the same region as97.2% (559/575) at1/200 titer with the use of same test (Guedes et al. 2008). Another study in Brazil in 2004 at Mato Grosso do Sul region (Andreotti et al. 2004) reported the seropositivity as 7.7% (7/91) with ELISA, while in another study in 2010 in the same region (Andreotti et al. 2010), the seropositivity was found to be 62.5% (687/1098) at 1/50 titre by IFA. When the studies in Turkey are concerned, a study in 2013 in Afyon province reported the rate of seropositivity as 21.03% (102/485) tested by c-ELISA (Çelik et al. 2013),while, in another study in 2016 in the same province, the rate of seropositivity was detected to be 3.8% (3/80) by in house ELISA (Zhou et al. 2016).

In the studies performed to find the seroprevalence of *N.caninum* in a specific region, it is significant to ensure the appropriate sample size and its representation of the region, to use same testing methods and cut-off values in regional and nation-wide studies as well as to consider such factors as species, breed, age, sex and various geographical parameters to reach accurate epidemiological data and for the control of the disease. In this study, a total of 225 cattle from 15 counties in Adana, with various climatic and geographic conditions, were grouped in respect of breed, age and sex; whilst the counties were grouped based on altitude. At the end of the study, through face-to-face interviews, the cattle owners were informed of the disease and its effect on their herds as well as the ways for its prevention and control.

The main purpose of cattle breeding is to obtain commercially valuable products such as meat, milk and leather from animals, and to get regularly one calf every year to ensure the continuity of the herds. Increasing feed and treatment costs affect farming negatively; and

sometimes, income from meat and milk barely covers the basic expenses. In such cases, the largest gain of the business is the offspring born healthy (Alpan 1994). In this context, abortions have become one of the most significant economic problems of the breeder (Juyal et al. 2011).

Nowadays, *N.caninum* is among the most important aborting agents (Hosseininejadet al. 2017). Serologic studies (c-ELISA, i-ELISA, IFAT) indicate that the prevalence of the disease is 11.3-59% in North America, 6.7-97.2% in South America, 1.5-43% in Asia, 0.5-80% in Europe and 3.4-19.64% in Africa (Table 2). Seroprevalence of *N.caninum* Turkey, on the other hand, was determined as 2-34.9% with ELISA test (Table 3).

According to the literature we examined, the mean seroprevalence of *N.caninum* is 13.06% (1023/7830) in Turkey (Table 3), 35.53% (1416/3985) in North America, 53.76% (4797/18922) in South America, 16.05% (1732/10787) in Asia, 23.69% (2681/11318) in Europe and 14.78% (598/4046) in Africa. The overall assessment of the mean seroprevalence in the world can be determined as 22.88% (11224/49058) (Table 2). In this study, we saw that the mean seroprevalence of Turkey, as a bridge between Asia, Europe and Africa, is lower than the mean seroprevalence of other continents and the world. At the end of the study, anti-*N.caninum* antibodies were detected in 10.7% of cattle in Adana province, and this rate is lower than the mean seroprevalence of Turkey and the world.

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