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Original Article

Investigation of *Neospora caninum* Seroprevalence and Association with Reproductive Problems in Cows in Burdur Province of Turkey

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Abstract

Background: An apicomplexan protozoon *Neospora caninum*, causative agent of neosporosis, is recognized as one of the most common and important cause of sporadic and endemic bovine abortion and reduced reproductivity in dairy and beef cattle worldwide. The present study was conducted to investigate the relationship between *N. caninum* seroprevalence and infertility problems in 400 cows in Burdur, city, Turkey.

Methods: Blood samples were collected from vena jugularis into sterile serum tubes from 49 aborted, 58 infertil, 48 pregnant and 245 healthy cows for the findings of reproductive anamnesis during a period of March 2010 to March 2011. Sera samples were analyzed by competitive ELISA kit.

Results: The seroprevalences were 7.7%, 6.4% and 4.2% in 2-4, ≤2 and ≥4 age groups respectively and no statistically significance observed between age groups. Seropositivity rates were 5.7%, 5.1%, 4.5%, 3.6% in Holstein, Montofon, cross-breeds and Simental breeds respectively. Seroprevalence differences was not statistically significant among cattle breeds. Antibodies to *N. caninum* were found in rates of 16.3%, 6.9%, 6.3%, 2.4% in aborted, infertile, pregnant and healthy cows respectively and there was a significant difference ($P<0.01$) between aborted and healthy animals. Seroprevalences were Yeşilova 10%, Gölhisar and Ağlasun 8%, Bucak, Çavdır and Kemer 4%, Karamanlı and Burdur Centrum 2%, according to districts.

Conclusion: The seroprevalence of *N. caninum* was revealed in Burdur region. It was emphasized that *N. caninum* infection should not be ignored in reproductive problems, especially in abortion cases.

Introduction

Neosporosis is regarded as a worldwide serious disease especially effects cattle and dogs, caused by *Neospora caninum*, which was first recognised in dogs (1) and then in aborted bovine fetuses (2, 3). *N. caninum* is an obligate intracellular and tissue cyst-forming apicomplexan parasite, has an heteroxenous life cycle between canids as definitive and a variety of warm-blooded animals as intermediate host. Clinical neosporosis has been reported in sheep, goat, deer and horses (4). It has emerged as a major cause of economic losses, generally related to abortion, stillbirth and other reproductive problems in most common intermediate host dairy and beef cattle all over the world (4, 5).

The horizontally transmission of *N. caninum* occurs by ingestion of tissue cysts or tissues infected with tachyzoites for definitive host canids and ingestion of food or drinking water contaminated by sporulated oocysts for cattle and other intermadiate hosts. Also it can be transmitted vertically (transplacentally or congenitally) to the fetus; as exogenous after ingestion of sporulated oocyst by pregnant dam or as endogenous in a chronically (persistently) infected dam by reactivation of the infection during pregnancy (6).

Generally neosporosis occurs latent and asymptomatic in non-pregnant cattle, however abortions caused by *N. caninum* can be observed from 3 months to term (often occurs at 5-6 months) at any age of cow sporadically, endemically or epidemically (4). *Neospora*-seropositive animals are 12.2 times more under risk of abortion than the seronegative ones (7). Also the abortion risk is significantly higher during the second term of gestation than the first and third terms (7). Altough clinically normal but persistently infected calves can be born, fetuses may die in utero, be mummified, autolyzed, absorbed, stillborn or born alive with some other clinical signs due to bovine neosporosis (4, 8). The transplacental transmission and clinically normal but persistently infected calves play an important role on spreading of the parasite and epidemiology of the disease (5). Control programs of bovine neosporosis are based on serodiagnosis and herd management, because effective treatments and vaccines are unavailable (9).

Necropsy, histopathological and immunohistochemical examinations of some tissues (brain, spinal cord, heart, liver, lung) of aborted fetus and several PCR-based methods can be used for diagnosis of *Neospora*-induced abortion (10). *Neospora*-induced abortion can also be confirmed by the observation of a statistically significant association between seropositivity and abortion within the group of dams with an abortion risk (10).

There are several serologic techniques such as indirect fluorescent antibody test (IFAT), various enzyme-linked immunosorbent assays (ELISAs), immunoblotting (IB) and direct agglutination tests (DATs) to detect specific antybodies against *N. caninum* in maternal blood to differentiate infected from non-infected animals (8). Serological assays provides a cost advantage compared to other tests. The most commonly commercialized serologic tests used for this purposes are ELISAs. Among the serological methods, ELISA is the most suitable for high throughput screening of antibodies against *N. caninum* (11), particularly for survey studies in large-scale herds (12). Hence, commercially available monoclonal antibody-based Competitive ELISA (cELISA) (13) was also used in the present study to detect of serum antibodies against *N. caninum* in cattle.

The present study was conducted to investigate the *N. caninum* seroprevalence in Burdur region and its relationship with reproductive problems such as abortion and infertility.

Materials and Methods

Study area, animals and blood samples

The study was based on data gleaned from a total of 400 cows from different ages and breeds from Burdur city center, Bucak, Aglasun, Yesilova, Kemer, Cavdir, Karamanli and Golhisar districts of Burdur province of Turkey. Blood samples were collected from vena jugularis into sterile serum tubes from 49 aborted, 58 infertil, 48 pregnant and 245 healthy cows for the findings of reproductive anamnesis. The cows examined were randomly selected. Serum has been obtained by removing after centrifugation of blood samples at 3000 rpm for 10 min and stored at -20°C up to the serological analysis were applied.

Serological test

The antibodies to *N. caninum* in sera samples were detected by using a commercially available competitive cELISA test kit (VMRD, Pullman, USA). The serological analysis were performed following the manufacturer's instructions. To determine the values for optical density (OD), a microplate reader (Bio-Tek Instruments, ELX800, USA) was used by seting at 620 nm wave length. The formula: Inhibition (%) = 100 - [(Sample O.D. X 100) ÷ (Mean Negative Control O.D.)] was used to assess the percent inhibitions of antibodies. According to the manufacturer; the samples with ≥ 30% inhibition values were considered as positive while with< 30% inhibition values were considered as negative.

Statistical analysis

Statistical analysis was performed using the Pearson chi-square test with the help of MiniTab16 Statistics program. The Chi-square test was applied to compare the prevalences of seropositive cows according to age, breed and reproductive anamnesis. The *P*-value is less than 0.05 considered as statistically significant.

Ethics Statement

Informed consents was taken from all animal owners. The study was approved by the Animal Experiments Local Ethics Committee of the Suleyman Demirel University (Protocol number: 07/04-2009).

Results

N. caninum seroprevalences in cattle in Burdur region are presented in Table 1, according to age, breed, districts and reproductive anamnesis. As seen in Table 1, no statistically significance was observed among seroprevalences of age groups and breeds. The seroprevalence was significantly higher in aborted than in non-aborted (total number of infertile, pregnant and healthy) cows. Also a statistically significance ($P<0.001$) were observed in the seroprevalence of *N. caninum* among aborted and healthy animals.

Discussion

N. caninum infection is admitted as one of the most common and important disease, cause sporadic and endemic bovine abortion and reduced reproductive efficiency in dairy and beef cattle worldwide (4, 14). An apicomplexan protozoon *N. caninum*, causative agent of neosporosis, is responsible for significant economic loses due to reproductive problems mainly abortion in all six continents (5).

In recent years, several epidemiological studies have been performed in different countries and the seroprevalence varied among dairy and beef cattle as well as in non-aborting and aborting populations of cattle. Seroprevalence of *N. caninum* has been reported in rates of 36.8% in Spain (15), 57.5-59% in Mexico (16, 17), 28-46% in Portugal (18), 5.6-7% in western Canada, 7.5-8.2% in Quebec and Ontario, 10.4-25.5% in Atlantic Canada and 9% in Northern Alberta (14), 5.7% in Japan (19), 15.2% in Greece (20), 40.3% in Romania (21), 13.81% in Brazil (22), 3.8% -26% in Iran (23, 24), 28.3-76.9% in Colombia (25, 26), 21.5% in Tanzania (27).

Table 1: Seroprevalence of *N. caninum* in cows in Burdur region with regard to origin, breed, age and reproductive anamnesis

Variable		*Examined* (no)	*Infected* (no)	*Prevalence* (%)
District				
	Merkez	50	1	2
	Aglasun	50	4	8
	Bucak	50	2	4
	Cavdir	50	2	4
	Karamanli	50	1	2
	Kemer	50	2	4
	Golhisar	50	4	8
	Yesilova	50	5	10
P-value				P>0.05
Breed				
	Holstein	283	16	5.7
	Simmental	56	2	3.6
	Montofon	39	2	5.1
	Crossbreed	22	1	4.5
P-value				P>0.05
Age (yr)				
	≤2	47	3	6.4
	2-4	91	7	7.7
	≥4	262	11	4.2
P-value				P>0.05
Reproductive anamnesis				
	Aborted	49	8	16.3[a]
	Infertil	58	4	6.9[ab]
	Pregnant	48	3	6.3[ab]
	Healthy	245	6	2.4[b]
P-value				P<0.001
Total		400	21	5.3

The difference between the indices shown with different letters (a, b) was statistically significant (*P*<0.05)

The seropositivity rates of *N. caninum* in various regions of Turkey were also reported as follows; Sakarya 9.2% (28), Trakya region 8.02% (29), Sanliurfa 7.5% (30), Kayseri 7% (31), Central Anatolia 13.96% (32), Elazig 8.19-15% (33,34), Van 4.88% (35), Mus 4.86%, Malatya 4%, Kars 2-7.2%, Bingol 4.69%(36,37), Kırıkkale 46.4% (38), Erzurum 10.65% (39), Afyon 21.03% (40), Nigde 26.51% (41), Kirsehir 18.1% (42), Adana 10.7% (43), Bursa 33.3% (44). In our study we determined 5.3% of seroprevalence for *N. caninum* in Burdur province. This rate is parallel to results of some studies (32, 33). However the seroprevalence was lower than results of some previous studies (28-31, 38) and higher than some others (33, 36). Results of the present and previous studies reveals the presence of *N. caninum* antibody in cattle in Turkey as the other countries of the world.

Only clinical symptoms are not enough for certain diagnosis of neosporosis. Also in aborted fetuses, the parasites may not be detected because of mostly autolysis of the tis-

sues. Therefore, *N. caninum* antibodies in serum and other body fluids should be determined for safer diagnosis and commonly serological tests such as IFAT, ELISA, DAT and IB can be used for this purpose. In addition, molecular assays as PCR are commonly used for diagnosis. ELISA test is often used to diagnose infections in large scale animal farms, because it provides faster and more reliable results than other serological tests (13, 14, 45). Therefore, commercial competitive inhibition ELISA test (c-ELISA) based on monoclonal antibody was preferred in the present study.

Some authors (46, 47) have reported that there is a relationship between seropositivity and age factor in neosporosis, although some authors (15, 30, 31, 33) have indicated that there is no relationship between seropositivity and age factor. In the present study, the highest prevalence rate was found as 7.7% (7/91) at the 2-4 age group but no statistically significant difference was determined between different age groups.

In epidemiological studies on neosporosis, there may be a relationship between seropositivity and cattle breeds, but it is stated that this is not caused by breed characteristics but by different breeding and accommodation conditions (15, 36, 46). Some authors (30, 33, 48) have reported that the differences between seroprevalence and cattle breeds is not statistically significant. Similarly, in the present study, the highest prevalence (5.7%) was found in Holstein cows but no statistically significance was found between seroprevalence and breeds.

Bovine neosporosis is considered as one of the major causes of epidemic and endemic abortions and economic losses worldwide (14, 20, 45). It is considered that this infection leads to a decrease in milk yield in dairy cattle due to its effect on fertility (49). *N. caninum* infection-related cow abortions and neonatal deaths have been recorded in some countries (17, 18, 48). Cows can abort at any time from the third month of pregnancy, but most of the abortions are about 5-6 months (4). Sadrebazzaz et al (48) reported a seroprevalence of 15.1% in the prevalence study of 810 cows in Iran and the seroprevalence in 139 aborted cows was found as 19.4%. Canada et al (18) found that the seroprevalence of 114 cows was 28% and found 46% of 1237 cows with abort story in the past. Another study (31) reported that in 13 (7%) of 186 cattle sera *N. caninum* antibodies detected in Kayseri region of Turkey. In the same study three of nine aborting cows (33.3%) were seropositive. The seropositivity differences among aborted and non-aborted cows were statistically significant ($P<0.05$). Aktas et al (33) found seropositivity in some Eastern Anatolia cases between 4-15%, and one of the 32 aborted cows (3.12%) was detected as *N. caninum* seropositive. Kul et al (38) found a prevalence of 46.47% in a study conducted in Kirikkale in a dairy cow operated with epidemic abortions. This rate was reported as 60% in cows with a problem of abortion and prolapse, 40% in heifers and 33.3% in calves.

In the present study, *N. caninum* antibodies were found as 16.3% (8/49) in aborted animals and the differences between healthy and aborted animals were found statistically significant ($P<0.01$). However, in order to be able to say precisely that the cause of abortion in these animals originated from *N. caninum*, it is thought that other causes of abortion should also be assessed and the pathogen agent from abortion material should be isolated. Nonetheless, the high seroprevalence in these animals suggests a strong association between abortion and *N. caninum*. The importance of this infection is not just because of abortion, also various infertility problems as early embryonic deaths, stillbirths and recurrent breeding problems can be caused by *N. caninum* infection (4, 14). Simsek et al (34) reported 13.48 % and 3.19% seroprevalence rates in animals with repetitive breeding problems and in healthy pregnant animals respectively. In the present study, seroprevalence in infertile animals was found as 6.9% (4/58) and this rate is above the overall prevalence.

In the present study, *N. caninum* seropositivity was 10% in Yesilova, 8% in Golhisar and Aglasun, 4% in Bucak, Cavdir and Kemer, and 2% in central district of Burdur and Karamanli. The geographical structure, ecological factors and definitive host population, which are important parameters in parasite biology, may be important in the difference seropositivity rates between the regions.

Conclusion

In the present study, the seroprevalence of *N. caninum* in cows in Burdur region was found as 5.3%. These results can give an idea of the existence of the parasite in this region. It was concluded that; the age and the breed of the animals are not important factors at the seropositivity rate, the high prevalence (16.3%) especially in the aborted animals should not be ignored. Moreover, isolation and molecular recognition of the agent from the aborted fetuses are beneficial for a safer diagnosis.

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Conflict of interest

The authors declared that there is no conflict of interest.

References

1. Bjerkas I, Mohn SF, Presthus J. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z Parasitenkd, 1984; 70(2):271-4.
2. Thilsted J P, Dubey J P. Neosporosis-like abortions in a herd of dairy cattle. J Vet Diagn Invest. 1989; 1(3):205-209.
3. Barr BC, Anderson ML, Blanchard PC, et al. Bovine fetal encephalitis and myocarditis associated with protozoal infections. Vet Pathol. 1990; 27(5):354-61.
4. Dubey JP, Schares G. Neosporosis in animals-The last five years. Vet Parasitol.2011; 180(1-2):90-108.DOI: 10.1016/j.vetpar.2011.05.031
5. Anderson ML, Andrianarivo AG, Conrad PA. Neosporosis in cattle. Anim Reprod Sci. 2000; 60-61:417-31.
6. Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and Control of Neosporosis and *Neospora caninum*. Clin Microbiol Rev.2007; 20(2):323-67.
7. Lopez-Gatius F, Lopez-Bejar M, Murugavel K, et al. *Neospora*-associated Abortion Episode over a 1-Year Period in a Dairy Herd in North-east Spain. J Vet Med B Infect Dis Vet Public Health. 2004; 51(7):348-52.
8. Ortega-Mora LM, Fernandez-Garcia A, Gomez-Bautista M. Diagnosis of bovine neosporosis: Recent advances and perspectives. Acta Parasitol. 2006; 51(1):1-14.
9. Alvarez-Garcia G, Garcia-Culebras A, Gutierrez-Exposito D, et al. Serological diagnosis of bovine neosporosis: a comparative study of commercially available ELISA tests. Vet Parasitol.2013; 198(1-2):85-95.
10. Dubey JP, Schares G. Diagnosis of bovine neosporosis. Vet Parasitol. 2006; 140(1-2):1-34.
11. Gondim LFP, Sartor IF, Hasegawa M. Seroprevalance of *Neospora caninum* in dairy cattle in Bahia, Brazil. Vet Parasitol.1999; 86(1):71-5.
12. Atkinson R, Harper PAW, Reichel MP, et al. Progress in the serodiagnosis of *Neospora caninum* infections of cattle. Parasitol Today. 2000; 16(3):110-4.
13. Baszler TV, Adams S, Schalie JV, et al. Validation of a commercially available monoclonal antibody-based competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to *Neospora caninum* in cattle. J Clin Microbiol. 2001; 39(11):3851-7.
14. Haddad JP, Dohoo IR, VanLeewen JA. A review of *Neospora caninum* in dairy and beef cattle-a Canadian perspective. Can Vet J. 2005; 46(3):230-43.

15. Quintanilla-Gozalo A, Pereira-Bueno J, Tabares E, et al. Seroprevalence of *Neospora caninum* infection in dairy and beef cattle in Spain. Int J Parasitol, 1999; 29(8):1201-8.
16. Vazquez ZG, Vazquez CC, Espinosa LM, et al. Serological survey of *Neospora caninum* infection in dairy cattle herds in Aquascalientes, Mexica. Vet Parasitol.2002; 106(2):115-20.
17. Gonzalez JJG, Cruz-Vazquez C, Esparza LM, et al. Management factors associated with seroprevalence to *Neospora caninum* infection in dairy cattle in Aguascalientes, Mexico. Vet Mexico.2007; 38:261-70.
18. Canada N, Carvalheira J, Meireles CS, et al. Prevalence of *Neospora caninum* infection in dairy cows and its consequences for reproductive management. Theriogenology. 2004; 62(7):1229-35.
19. Koiwai M, Hamaoka T, Haritani M, et al. Nationwide seroprevalence of *Neospora caninum* among dairy cattle in Japan. Vet Parasitol. 2006; 135(2):175-9.
20. Sotiraki S, Brozos C, Samartzi F, et al. *Neospora caninum* infection in Greek dairy cattle herds detected by two antibody assays in individual milk samples. Vet Parasitol.2008; 152(1-2):79-84.
21. Mitrea IL, Enachescu V, Radulescu R, et al. Seroprevalence of *Neospora caninum* infection on dairy cattle in farms from southern Romania. J Parasitol.2012; 98(1):69-72.
22. Padilha MAC, Wasen G, Souza AP, et al. *Neospora caninum*: Seroprevalence in beef cattle in the mountainous region of Santa Catarina, Brazil. Semin Cienc Agrar.2017; 38(1):273-82.
23. Norollahi-Fard SR, Khalili M, Fazli O, et al. Seroprevalence of *Neospora caninum* in cattle of Neishabour, Northeast Iran. Slov Vet Res.2017; 54(1): 5-9.
24. Noori M, Rasekh M, Ganjali M, et al. Seroprevalence of *Neospora caninum* infection and associated risk factors in cattle of Sistan areas, Southeastern Iran in 2016. Iran J Parasitol.2019; 14(2): 340-6.
25. Cedeno QD, Benavides BB. Seroprevalence and risk factors associated to *Neospora caninum* in dairy cattle herds in the municipality of Pasto, Colombia. Rev MVZ Cordoba.2013; 18(1):3311-6.
26. Llano HAB, Guimaraes MS, Soares RM, et al. Seroprevalence and risk factors for *Neospora caninum* infection in cattle from the eastern Antioquia, Colombia. Vet Anim Sci. 2018; 6:69-74.DOI: 10.1016/j.vas.2018.03.001
27. Semango G, Hamilton CM, Kreppel K, et al. The Sero-epidemiology of *Neospora caninum* in Cattle in Northern Tanzania. Front Vet Sci.2019; 6:327.
28. Oncel T, Biyikoglu G. Neosporosis in dairy cattle in Sakarya, Turkey. J Res Vet Med. 2003; 22:87-9.
29. Biyikoglu G, Bagci O, Oncel T.Serological Survey of *Neospora caninum* Infection. Indian Vet J.2005; 82(3):345-6.
30. Sevgili M, Altas MG, Keskin O. Seroprevalence of *Neospora caninum* in cattle in the province of Sanliurfa. Turkish J Vet Anim Sci, 2005; 29:127-30.
31. Ica A, Yildirim A, Duzlu O, et al. Seroprevalence of *Neospora caninum* in Cattle in the Region of Kayseri. Turkiye Parazitol Derg.2006; 30(2):92-4.
32. Vural G, Aksoy E, Bozkir M, et al. Seroprevalence of *Neospora caninum* in dairy cattle herds in Central Anatolia, Turkey. Vet Arh.2006; 76:343-9.
33. Aktas M, Saki CE, Altay K, et al. Survey of *Neospora caninum* in cattle in some provinces in the Eastern Anatolian region. Turkiye Parasitol Derg.2005; 29(1):22-5.
34. Simsek S, Utuk AE, Koroglu E, et al. Seroprevalence of *Neospora caninum* in repeat breeder dairy cows in Turkey. Arch Tierz.2008; 51(2):143-8.DOI: 10.5194/aab-51-143-2008
35. Alan M, Cetin Y, Sendag S, et al. Seroprevalence of antibodies against *Neospora caninum* in cows in Van province. Kafkas Univ Vet Fak Derg.2011; 17(5):767-71.
36. Akca A, Gokce HI, Guy CS, et al. Prevalence of antibodies to *Neospora caninum* in local and imported cattle breeds in the Kars province of Turkey. Res Vet Sci. 2005; 78(2):123-6.DOI: 10.1016/j.rvsc.2004.08.006
37. Mor N, Akca A. Kars Yöresinde sığır ve köpeklerde *Neospora caninum* üzerine epidemiyolojik araştırmalar: Gruplararası çalışma. Kafkas Univ Vet Fak Derg. 2012; 18(Suppl-A), A193-9.DOI: 10.9775/kvfd.2012.6181
38. Kul O, Kabakci N, Yildiz K, et al. *Neospora caninum* associated with epidemic abortions in dairy cattle: the first clinical neosporosis report in Turkey. Vet Parasitol.2009; 159(1):69-72.DOI: 10.1016/j.vetpar. 2008.10.019

39. Balkaya I, Bastem Z, Avcioglu H, et al. Seroprevalence of *Neospora caninum* Antibodies in Cattle in Eastern Turkey. Isr J Vet Med.2009; 67(2):109-12.
40. Celik HA, Kozan E, Eser M, et al. A research on seroprevalence of *Neospora caninum* in cattle. Ankara Univ Vet Fak Derg, 2013; 60:99-102.DOI: 10.1501/Vetfak_0000002561
41. Karatepe B, Karatepe M. Seroprevalence of *Neospora caninum* in cattle in Nigde Province, Turkey. Isr J Vet Med.2016; 71:39-42.
42. Yildiz K, Gokpinar S, Sursal N, et al. Seroprevalence of *Neospora caninum* in Dairy Cattle Raised in Cicekdagi District of Kirsehir Province. Turkiye Parasitol Derg.2017; 41(3):135-8.DOI: 10.5152/tpd.2017.5218
43. Eski F, Utuk AE. Detection of Anti-*Neospora caninum* Antibodies in Cattle in Adana Province of Turkey. Van Vet J. 2018; 29(2):93-9.
44. Kasap S, Ertunc S, Temizel EM, et al. A study of *Neospora caninum* antibody seroprevalence in dairy cows in Turkey. J Hellenıc Vet Med Soc, 2020; 71:2019-22.DOI: 10.12681/jhvms.22950
45. Wouda W, Moen AR, Schukken YH. Abortion risk in progeny of cows after a *Neospora caninum* epidemic. Theriogenology.1998; 49(7):1311-6.DOI: 10.1016/S0093-691X(98)00078-8
46. Thurmond MC, Hietala SK. Culling associated with *Neospora caninum* in dairy cows. Am J Vet Res.1996; 57:1559-62.
47. Sanderson MW, Gay JM, Baszler TV. *Neospora caninum* seroprevalence and associated risk factors in beef cattle in the Northwestern United States. Vet Parasitol.2000; 90:15-24.DOI: 10.1016/s0304-4017(00)00234-x
48. Sadrebazzaz A, Haddadzadeh H, Esmailnia K, et al. Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. Vet Parasitol. 2004; 124: 201-4. DOI: 10.1016/j.vetpar.2004.06.027
49. Dumanli N, Aktas M. Toxoplasmatidae (*Toxoplasma*, *Neospora*). In: Dumanli N, Karaer Z, editors. Veteriner Protozooloji. Ankara; Medisan:2010. p. 128-35.