Kafkas Univ Vet Fak Derg
17 (5): 767-771, 2011
DOI:10.9775/kvfd.2011.4415

RESEARCH ARTICLE

Seroprevalence of Antibodies Against *Neospora caninum* in Cows in Van Province [1]

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[1] *This study was funded by Yuzuncu Yil University, Scientific Research Projects Fund (Project No: 2007-VF-B06)*
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Makale Kodu (Article Code): KVFD-2011-4415

Summary

The objective of this study was to determine the seroprevalence of *Neospora caninum* by cELISA among cows in Van province in Turkey. As material; 450 cows consisting of Brown Swiss, Simmental, Holstein, native species and crossbreds of these and given birth before at least one time were used. Approximately 7 ml blood sample was drawn from the jugular vein of each animal into the plain tubes. Sera obtained from the blood samples were stored at -20°C and processed using a commercial *N. caninum* antibody test kit (cELISA; VMRD, Inc.) after two mounts from the last sampling. Inhibitions ≥30% were interpreted as seropositive and inhibitions <30% were interpreted as seronegative. Seroprevalence of antibodies against *N. caninum* in cows in Van province was 4.88% as a general average. Seropositive sample rate of the central districts was higher than the rate of central villages, 10.66% vs. 3.73%. On the other hand, seropositive sample rate of the animals came to the animal hospital from the central villages was higher than the rate of animals sampled in their places, 8.06% vs. 2.87%. In conclusion, the seroprevalence of *N. caninum* among cows in Van province in Turkey is lower or higher than those of some other provinces in Turkey and countries or regions in the world. The reason of this may be different cattle breeds, breeding systems and animal traffic among regions or countries. Along with main control measurements to contain *N. caninum* infection, diagnosing and culling of seropositive animals would give helpful results.

Keywords: *Neospora caninum, Seroprevalence, Cow, Van*

Van Yöresinde İneklerde *Neospora caninum* Antikorlarının Seroprevalansı

Özet

Bu çalışmada Van yöresindeki ineklerde *Neospora caninum* seroprevalansının cELISA ile belirlenmesi amaçlanmıştır. Çalışmanın materyali olarak; Yerli Irklar, Holştayn, Simental, Esmer ve bunların melezlerinden oluşan ve önceden en az bir kez doğum yapmış 450 inek kullanıldı. Her bir hayvanın jugular venasından cam tüplere yaklaşık 7 ml kan örneği alındı. Kan örneklerinden elde edilen serumlar -20°C de depolandı ve son örnekleme işleminden iki ay sonra ticari bir *N. caninum* antikor test kiti (cELISA; VMRD, Şti.) kullanılarak işlendi. Inhibisyon değeri ≥%30 olanlar seropozitif ve <%30 olanlar seronegatif olarak yorumlandı. Genel bir ortalama olarak Van yöresinde ineklerde *N. caninum* antikorlarının seroprevalansı %4.88 bulundu. Seropozitif örnek oranı merkez mahallelerinde (%10.66) merkez köylerinden (%3.73) daha yüksek idi. Diğer yandan, seropozitif örnek oranı merkez köylerden hayvan hastanesine gelen hayvanlarda (%8.06) köylerde, yerinde, örnek alınan hayvanlara göre (%2.87) daha yüksek olarak elde edildi. Sonuç olarak, Van yöresinde ineklerde *N. caninum* antikorları seroprevalansının Türkiye'deki bazı iller ve dünyadaki bazı ülke veya bölgelerden düşük veya yüksek olduğu görülmektedir. Bunun nedeni farklı sığır ırkları, yetiştirme sistemleri ve bölgeler ya da ülkeler arası hayvan trafiği olabilir. *N. caninum* enfeksiyonunu kontrol altına almak için esas kontrol yöntemleriyle birlikte, seropozitif hayvanların tanısı ve sürüden uzaklaştırılması yararlı sonuçlar verebilir.

Anahtar sözcükler: *Neospora caninum, Seroprevalans, İnek, Van*

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INTRODUCTION

Neospora caninum is an intracellular protozoan parasite of class coccida [1,2] and recognized worldwide, in particular in dairy cattle [3-7]. *Neospora caninum* was misdiagnosed as Toxoplasma gondii until 1988 because of morphological similarities. After the first report as a cause of abortion in cattle, neosporosis has become a growing concern in dairy and beef cattle reproduction due to important economic losses [4]. It has been associated with sporadic, endemic and epidemic abortions [1,2,8,9]. Before the complete life cycle of the parasite was known, findings in epidemiological studies indicated that the presence of dogs on a farm, as the definitive host, increased the risk of *N. caninum* abortion in cattle. Dogs infected experimentally or under natural condition were shown to excrete *N. caninum* oocysts with their faeces. Until recently the dog was the only species verified to be a definitive host of the parasite [1]. However, coyotes (*Canis latrans*) have been shown to be an additional definitive host by the other researchers [1,9]. It is assumed that there may be other definitive hosts in wild-life and some common species have been investigated. For example, foxes have been shown to carry the parasite but it has not been verified that foxes shed oocysts, i.e. are actual definitive hosts [1].

Transplacental or vertical transmission of the parasite from an infected dam to its fetus is the major natural route of infection [9-12]. Cattle may also acquire *N. caninum* infection by horizontal (postnatal) infection through the ingestion of oocysts shed in the feces of the definitive hosts [1,2,9]. There is a study [13] showing intermittent presence of *N. caninum* in blood and semen and shedding in semen in low numbers. Intrauterine inoculation via contaminated semen cause *N. caninum* infection in cattle [14]. Although some infected cows abort, many fetal infections produce a congenitally infected calf. A congenitally infected heifer calf is capable of transmitting the infection onto the next generation when she becomes pregnant, thus maintaining the infection in the herd [8,9,12].

The risk of abortion was 12.2 times higher in the *Neospora*-seropositive animals than in seronegative animals and significantly higher during the second term of gestation than during the first and third terms [15]. *N. caninum* infection prior to pregnancy appears not to affect the fertility [16] and early fetal period, but does have a significant abortive affect after 90 days of gestation in dairy cows [17,18]. The incidence of *N. caninum*-associated abortion peaks during the fifth to the seventh month of gestation [19]. *N. caninum* infection fails to affect early gestation because the tachyzoites have not enough time for replication to cause foetal death. Nevertheless, it was pointed out that in fetuses from the first period, the *N. caninum* infection was severe with high parasite loads and important lesions. It could, therefore, be speculated that fetal death could be a consequence of the parasite invasion [20].

There is some evidence that the epidemiology of neosporosis varies in dairy and beef cattle. Several studies have shown a lower prevalence of infection in beef cattle compared with dairy herds. The use of beef bull semen can reduce the risk of abortion in dairy cows, and annual screening for neosporosis, specifically the antibody titre to the protozoon, could be a useful predictor of abortion risk in reproductive health programes [19]. Vertical transmission of infection is the predominant mode of infection and hence control efforts aimed at selectively culling seropositive animals from the herd are highly successful in reducing the level of infection [21]. Repeated iscom ELISA test applied on tank bulk milk at regular intervals seems to be helpful and cost-effective for large epidemiological surveys, for monitoring control strategy plans for *N. caninum*, and for increasing the bio-safety level in dairy cattle farms [22,23].

Although *N. caninum* is an important abortifacent agent and studied intensively in the world, only a few studies conducted in Turkey [24-28] were encountered.

The objective of this study was to determine the seroprevalence of *N. caninum* by cELISA among cows in Van province in Turkey.

MATERIAL and METHODS

As material; 450 cows consisting of Brown Swiss, Simmental, Holstein, native species and crossbreds of these and given birth before at least one time were used. Three hundred and thirteen blood samples were collected from the cows in their stables in the villages. Sixty two blood samples were collected from the cows came from 38 villages of Van province to the animal hospital with any health issue. Remaining 75 blood samples were obtained from the animals bred in the stables in the central districts of Van Province. These samples were collected when the animals came to the animal hospital with any health issue. A registration sheet was filled in for every animal included.

Approximately 7 ml blood sample was drawn from the jugular vein of each animal into the plain tubes. Sera obtained from the blood samples were stored at -20°C and processed using a commercial *Neospora caninum* antibody test kit (cELISA; VMRD, Inc.) after two mounts from the last sampling. The commercial cELISA tests were performed according to the instructions of the manufacturers. Inhibitions ≥30% were interpreted as seropositive and inhibitions <30% were interpreted as seronegative.

The prevalence of *N. caninum* antibodies was expressed as percentage of samples considered positive with test used to the total samples examined. The prevalences of *N. caninum* antibodies among different sampling places or villages were compared using Chi-square analysis [29].

RESULTS

Seroprevalences of antibodies against *Neospora caninum* in cows in Van province are shown in *Table 1* as a whole. The results are detailed in *Table 2* and *Table 3*. Seropositive sample rate of the central districts was higher than the rate of central villages, 10.66% versus 3.73%. On the other hand, seropositive sample rate of the animals came to the animal hospital from the central villages was higher than the rate of animals sampled in their places, 8.06% versus 2.87%.

Reliable case histories of the 6 from 22 *Neospora caninum* seropositive cows during sampling were not available. Case histories of the other 16 cows are shown in the *Table 4*.

Table 1. *Seroprevalences of antibodies against Neospora caninum in cows in Van province*
Tablo 1. *Van yöresindeki ineklerde Neospora caninum antikorlarının seroprevalansı*

Sampling Place	Sample Number (n)	Seropositive Sample Number (n)	Seropositive Sample Rate (%)
Central villages of Van province (sampling in the places) [1]	313	9	2.87 a
Central villages of Van province (sampling in the animal hospital from cows came from 38 villages except the villages above) [2]	62	5	8.06 b
Central districts of Van province (sampling in the animal hospital)	75	8	10.66 c
Total	**450**	**22**	**4.88**

[1] Detailed in Table 2, [2] Detailed in Table 3, Table 3; a, b, c: $X^2 = 9.45$ *and* $P<0.01$

Table 2. *Central villages of Van province sampled in the places, sample numbers, seropositive sample numbers and rates*
Tablo 2. *Yerinde örnek alınan Van merkez köyleri, örnek sayıları, seropozitif örnek sayı ve oranları*

Villages	Sample Number (n)	Seropositive Sample Number (n)	Seropositive Sample Rate (%)
1. Bardakci (central districts)	24	1	4.16 a
2. Bardakci (Gariptepe disrict)	29	0	0 b
3. Hidir	116	2	1.72 c
4. Atmaca	84	3	3.57 d
5. Otluca	60	3	5 e
Total	**313**	**9**	**2.87**

a, b, c, d, e: $X^2 = 2.66$ *and* $P>0.05$

Table 3. *Central villages of Van province sampled in the animal hospital, sample numbers, seropositive sample numbers and total seropositive sample rate*
Tablo 3. *Hayvan hastanesinde örnek alınan Van merkez köyleri, örnek sayıları, seropozitif örnek sayıları ve toplam seropozitif örnek oranı*

Villages	Sample Numbers (n)	Seropositive Sample Numbers (n)	Villages	Sample Numbers (n)	Seropositive Sample Numbers (n)	Seropositive Sample Rate (%)
1. Agzikara	3	1	20. Hizir	1	0	
2. Sakalar	1	0	21. Topaktas	3	0	
3. Yemlice	2	0	22. Kumluca	2	0	
4. Asagi Ciftlik	2	0	23. Donerdere	1	0	
5. Golgecik	1	0	24. Gulsunler	2	0	
6. Gedikbulak	3	0	25. Gollu	6	2	
7. Yumrutepe	1	0	26. Ocaklı	1	0	
8. Turgali	1	0	27. Aktas	1	0	
9. Degirmenozu	1	0	28. Ugurveren	1	0	
10. Kurubas	1	0	29. Yolasan	1	0	
11. Kiratli	1	1	30. Dagini	1	0	
12. Citoren	5	0	31. Ermisler	1	0	
13. Karagunduz	1	0	32. Tevekli	1	1	
14. Ilikkaynak	2	0	33. Colpan	1	0	
15. Kigcak	1	0	34. Gulyazi	1	0	
16. Agarti	1	0	35. Kasimoglu	4	0	
17. Enginsu	1	0	36. Ulusar	1	0	
18. Dibek	1	0	37. Karaca	1	0	
19. Pirgarip	2	0	38. Gunbasi	1	0	
Total				**62**	**5**	**8.06**

Table 4. *Case histories of Neospora caninum seropositive cows obtained during sampling*

Tablo 4. *Neospora caninum seropozitif hayvanların örnekleme sırasında elde edilen anamnez bilgileri*

Cows	Case Histories
1	Approximately 4.5-5 months pregnant
2	Approximately 3-3.5 months pregnant
3	Infertile
4	Aborted two times in last pregnancies
5	Normal birth 3 days ago
6	Abort and infertility
7	Approximately 6-6.5 months pregnant
8	Abort
9	Approximately 8.5 months pregnant
10	Infertile
11	Normal birth 2 months ago
12	Normal birth 6 months ago
13	Normal birth 5 months ago
14	Normal birth 4 days ago
15	Normal birth 6 days ago and retained fetal membranes
16	Normal birth 1 month ago

DISCUSSION

Since the discovery of *N. caninum,* many diagnostic tests have been developed to help in diagnosing this parasitic infection [5]. The diagnosis of *N. caninum* induced abortion in individual cattle is based upon examination of fetal tissues for histological lesions, for tachyzoites by immunohistochemistry, or for parasite DNA by PCR. Validated *N. caninum*-specific serological assays are necessary for accurate herd-based abortion diagnosis and for population-based epidemiological investigations of disease transmission, disease risk factors, and identification of additional definitive and intermediate hosts [30]. The development of serological tests for *Neospora* infection fills an urgent need in the study of neosporosis. Because the complete host range and life cycle of *N. caninum* are unknown, antemortem serological tests specifically identifying *Neospora*-infected animals provide a valuable tool for epidemiologic and diagnostic investigations. Furthermore, the accurate identification of *Neospora*-infected cattle has important implications in the control of abortion since repeated abortion and congenital transmission can occur in some infected cows [31]. In addition, serological testing provides a competitive cost advantage over other tests. Of the different serological assays, ELISA is the most suitable for high throughput screening of antibodies to this parasite [32]. Competitive inhibition ELISA (cELISA), also used in this study, is unreactive to antigens of 2 closely related apicomplexan protozoa *Toxoplasma gondii* and *Sarcocystis cruzi*. This ELISA test has been adopted by many laboratories in Canada as the test of choice for detecting antibody against *N. caninum* [5].

Many cow-level and herd-level seroprevalences of *N. caninum* are reported from different countries of the world. There is some evidence that the epidemiology of neosporosis varies in dairy and beef cattle. Several studies have shown a lower prevalence of infection in beef cattle compared with dairy herds [19].

In dairy cattle; the cow-level seroprevalences ranged from 5.6% to 7.0% in western Canada, from 7.5% to 8.2% in Quebec and Ontario, from 10.4% to 25.5% in Atlantic Canada [5]. The rates of 5.7% and 12% were obtained from nationwide of Japon [33] and Parana State of Brazil respectively [4]. Individual dairy cattle prevalence of *N. caninum* was reported as 15.2% (by ELISA) in Greek. The prevalence varies between 0.5% and 2% in Sweden and 16.2-36% in Spain [7]. The herd-level seroprevalence in Australia and New Zealand is usually about 30 to 35% [3]. Herd prevalences in dairy cattle in European countries were reported to be 16% in Sweden, 49% in Germany, 63% in Spain and 76% in The Netherlands [7]. Seroprevalence rates of *N. caninum* of about 50 to 60% have been reported in dairy herds in Quebec [31]. In beef cattle; cow-level seroprevalences were reported as 11.5% for 1980 and 9% for 1998 in northern Alberta [5].

Herd-level blood sampling could not be possible because of many reasons in this study. Samples were collected wherever possible; in stables, in pastures or in the animal hospital. The cows randomly sampled were from combined, meet and milk, yielder. Obtained general cow-level seroprevalence rate of *N. caninum* of 4.88% is seen as near to or lower than the rates given above for other countries, except for Sweden. If it is compared to the other some provinces in Turkey, the seroprevalence rate of 4.88% in Van province is about the same as the rates reported [25] for Malatya (4%), Mus (4.86%) and Bingol (4.69%) but lower than the rates of 7% for Kayseri [27], 9.2% for Sakarya [24] and 15% for Elazıg [25]. There is an interesting report [28] that states a seropositive rate of 8.2% for imported Simmental cows and their offspring but no seropositivity in recently aborted local cow breeds in Kars province of Turkey.

The reason for higher seropositive animals (8.06% and 10.66%) sampled in the animal hospital than seropositive animals (2.87%) sampled in the villages could be that some health problems causing the animals being led to the animal hospital, in fact, might be related to *Neospora caninum* infection. It was difficult to take a decision where dog population, thought as a disseminating factor of the infection, was higher.

Case histories of *Neospora caninum* seropositive cows *(Table 4)* are similar to those explained in the former reports [2,5,7,12].

In conclusion, the seroprevalences of *N. caninum* among cows in Van province in Turkey are lower or higher than those of some other countries or regions in the world. The reason of this may be different cattle breeds, breeding systems and animal traffic among regions or countries. Along with main control measurements to contain *N.*

caninum infection, diagnosing and culling of seropositive animals would give helpful results.

Acknowledgement

The authors express their appreciations and thanks for the financial support of Scientific Research Projects Fund, Yuzuncu Yil University.

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