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Association between presence of DNA and antibody in the serum during vertical transmission of *Neospora caninum*

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Abstract

Neospora caninum is an intracellular parasite causing abortion and reproductive failure in cattle. The aim of this study was to determine the association between serum parasitemia and seropositivity in cows with no sign of abortion and their full term calves. For this purpose, 49 serum samples of normal full term delivering dairy cattle and their precolostral new born calves were tested by using PCR, nested-PCR and a new developed whole cell-based ELISA. Fourtheen of 49 mothers (28.57%) and 6 of 49 calves (12.24%) showed anti-*Neospora* serum antibodies and *Neospora* DNA, concurrently. All infected calves were born from infected mothers and the vertical rate of transmission among all samples was 6 out of 49 (12.24%) and from infected mothers 6 out of 14 (42.84%), based on different serum analyses. Eight out of 14 calves (57.14%) born from infected mothers were *Neospora* free. In conclusion, despite the presence of anti-*Neospora* serum antibodies in infected cows and calves, the parasite was not completely removed from the blood and so it seems that the presence of antibodies is not necessarily a sign of effective immunity.

Key words: *Neospora caninum*, Antibodies, DNA

Introduction

Neospora caninum is an obligate intracellular parasite (Dubey, 2003), which is accounted for as a major cause of reproductive failures. During the second and third semester of the pregnancy, the infected animals may deliver either preterm or apparently healthy born (AHB) calves (Dubey and Schares, 2006). As for other pathogenic agents, *Neospora* is recognized by the fetal immune system and controlled by the fetal immune system; thereafter the abortion decreased after the first trimester transplacental tranmission. These pregnant mothers may deliver AHB calves which were vertically infected with the parasite and may pose various pathologic foot prints on the fetal tissues (Dubey, 2003).

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The essential role of AHB calves in keeping and spreading the infection among the population has been discussed in some recent studies. The role of dogs, as the definitive host in industrial livestock is almost controlled; therefore, the main root of transmission for *Neospora* in cattle of industrial farms is congenital (Santos et al, 2012). In these farms, AHB calves remain infected during their whole life transferring the parasite to their offsprings (Nasir et al, 2012).

As a matter of fact, cell-mediated immunity (CMI) plays a major role in protection of *N. caninum* as an obligate intracellular parasite (Guy et al, 2001; Paré et al, 1997; Stenlund et al, 1999). Increased serum antibodies may be used as an indirect indicator of parasite multiplication, whether antibodies can directly influence recrudescence (Guy et al, 2001; Paré et al, 1997; Stenlund et al, 1999). The present study was designed to evaluate the association of anti-*Neospora* antibody and the possible presence of *Neospora* DNA in the serum of pregnant cows and their AHB precolostral calves.

Materials and Methods

Samples

Blood samples were obtained from 49 full term pregnant cows immediately after delivery and their AHB calves before receiving their colostrum in an industrial dairy farm. The samples were taken from the jugular vein using venoject plain tubes with no anti-coagulants. The tubes were centrifuged at 1000g for 10 minutes and the sera were collected and kept at -20 until used.

ELISA

ELISA plates (Biofil, Canada) were coated with 2×10^6 *Neospora* tachyzoites per well and incubated at room temperature for 3 days. The plates were washed with a washing buffer containing PBS and 0.05% Tween 20 for three times and blocked with a blocking buffer containing PBS and marvel milk 5% for 1h at 37˚C. The serum samples were loaded onto the ELISA plates in duplicate after a dilution of 1 in 100 in the blocking buffer and incubated at 37˚C for 1 h followed by three times washes. A number of serum samples collected from *Neospora* positive cows were used as positive controls and the blocking buffer with no serum was used as the negative control. HRP conjugated sheep anti-bovine IgG–heavy chain antibody (Bethyl, USA) was diluted in PBS (1:1000) containing 5% marvel milk and added to the plates incubating at 37˚C for 1 h. After three times washes with the washing buffer, a substrate containing DMSO, TMB 10 µg/ml, sodium acetate 0.1% and hydrogen peroxide10% (all from Sigma-Aldrich, USA) was added and the plates were incubated again at room temperature for 40 minutes. 2M H_2SO_4 was used to stop the reaction and the plates was then read at 450nm on a micro-plate ELISA reader (ELX808, Bio Tec, USA). The ratio of sample/positive control (S/P) ODs was calculated according to the following equation.

$$S/P = \frac{\text{sample} - \text{NC}}{\text{PC} - \text{NC}}$$

NC: Negative Control
PC: Positive Control

Samples with the S/P ratio of 0.50 or above were considered as positive for *N. caninum* infection (Hajikolaei, Goraninejad, Hamidinejat, Ghorbanpour, & Paryab, 2007; Yu et al., 2007).

PCR

N.caninum NC5 gene was detected in serum samples using Np6 forward (5′-CTCGCCAGTCAACCTACGTCTTCCT>-3′) and Np21 reverse (5′-CCCAGTGCGTCCAATCCTGTAACC>-3′) primers (Müller, Zimmermann et al. 1996). The PCR was programmed as 10 minutes at 95˚C for primary denaturation and 35 cycles of 95˚C for 1 minute, 65°C 1 min and 72°C for 2 minutes and a final extension at 72°C for 10 min.

Nested-PCR

1 µl of the PCR products was subjected to nested-PCR for conforming the presence of *N.caninum* NC5 gene using 5'-GTGTTGCTCTGCTGACGTGT-3' forward and 5'-TACCAACTCCCTCGGTTCAC-3' reverse primers. The nested PCR was programmed as 10 minutes for primary denaturation at 95°C and 35 cycles of 1 minute at 95°C for denaturation, 45 seconds at 54°C for annealing and 1 minute at 72°C for extension. Finally, the reaction was completed with a final extension at 72°C for 10 minutes.

Statistical Analysis: Statistical analysis of the relationship and correlation coefficient between nominal variables was performed by Chi-square and Phi correlation coefficient, respectively. Data normality was checked out by Kolmogorov Smirnov method. Pearson's correlation coefficient was done for s/p data. Data analyses were performed at the α=0.05 level by SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and MedCalc 20.1.4.

Results

Anti-*Neospora* antibodies were detectable in 14 mothers (28.57%) and 6 calves (12.24%) (Table 1). A significant positive correlation was observed between the serum ELISA titer (S/P ratio) of mothers and calves (r=0.351, n=49, *p*-value=0.013). Cows and new born calves with positive serum PCR showed a high S/P ratio of ELISA titer (Table 3).

Based on PCR and Nested-PCR findings (Figures 1 & 2), there was a significant relationship between maternal and offspring infection (r=0.591, n=49, *p*-value=0.000036), (Tables 1 & 2). The rate of vertical transmission was 42.84% (6 infected calves out of 14 infected dams); the overall rate of vertical transmission from all mothers to the infected foetuses was 12.24% (6 infected calves out of 49 total dams). Impressively, some of the calves born from infected mothers were *Neospora* free and all infected calves were born from infected mothers (Table 2). The results of PCR and Nested-PCR were completely consistent with each other.

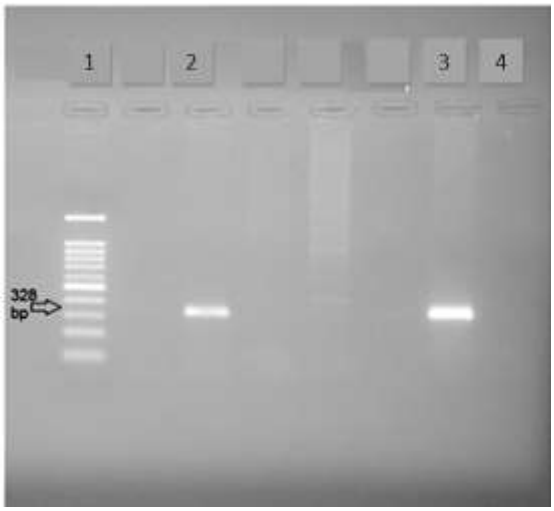

Figure 1. Detection of *Neospora caninum* DNA in bovine serum; 1: ladder 2: serum samples 3: positive control (*Neospora* DNA) 4: negative control

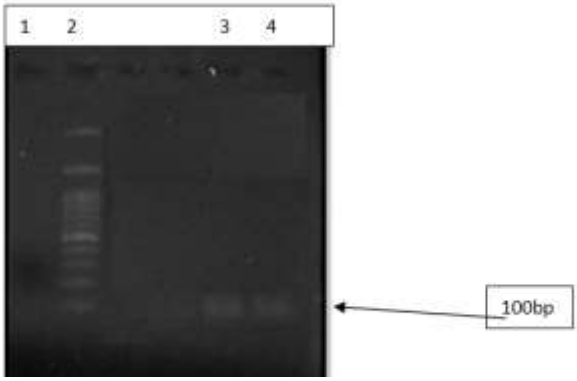

Figure 2. Confirmation of *Neospora caninum* **NC5 gene in PCR product by nested PCR;1: Negative Control 2: Standard DNA 3: Cow serum samples 4: Calf serum samples.**

Table 1: Detection of anti-*Neospora caninum* **antibodies and** *Neospora caninum* **DNA by ELISA, PCR and nested-PCR***

	Caws				Calves			
Sample No.	S/P **	ELISA	PCR	nested-PCR	S/P **	ELISA	PCR	nested-PCR
1	0.842	+	+	+	0.706	+	+	+
2	0.524	+	+	+	0.021	_	_	_
3	0.56	+	+	+	0.42	_	_	_
4	0.669	+	+	+	1.032	+	+	+
5	0.911	+	+	+	0.141	_	_	_
6	0.572	+	+	+	0.576	+	+	+
7	1.197	+	+	+	0.706	+	+	+
8	0.588	+	+	+	1.26	+	+	+
9	0.685	+	+	+	1.44	+	+	+
10	0.834	+	+	+	0.228	_	_	_
11	0.649	+	+	+	0.04	_	_	_
12	0.766	+	+	+	0.108	_	_	_
13	0.991	+	+	+	0.108	_	_	_
14	0.508	+	+	+	0.347	_	_	_
Total Positive (%)	0.73±0.19	14 (28.57)	14 (28.57)	14 (28.57)	0.51±0.44	6 (12.24)	6 (12.24)	6 (12.24)

**: Cows with S/P ELISA titer greater than 0.5, PCR or nested PCR were considered positive (n=14); S/P: OD of standard samples to positive samples ; **: Mean±Standard Deviation.*

Table 2: Infection status of mothers and its relationship with maternal infection of calves (n=49)*

	Positive calves	Negative calves	Vertical Transmission Rate (%)	Relative Risk (CI95%)
Positive Dam	6	8	42.85%	31.2 (1.9-519.6)
Negative Dam	0	35	0%	
Total	6	43	12.24%	
p-value	0.000036			0.016

**: Cows with S/P ELISA titer greater than 0.5, PCR or nested PCR were considered positive (n=49); CI95%: 95% confidence interval*

Table 3: S/P ELISA titer status of infected mothers and their calves (n=14)*

	calf		
	PCR positive (n=6)	PCR negative (n=8)	*p*-value
Dam S/P	0.76±0.23	0.72±0.18	0.732
Calf S/P	0.95±0.35	0.18±0.14	0.002

**: Calves with S/P ELISA titer greater than 0.5, PCR or nested PCR were considered positive; S/P: OD of standard samples to positive samples ; **: Mean±Standard Deviation.*

Discussion

Based on serum *Neospora* genomic findings, statistical analysis showed a significant relationship (*p*-value=0.000036) between maternal infection and the possibility of newborn infection (Table 2). Transplacental transmission rate observed in 12.24% of all cows and 42.85% of positive cows. The first report on the abortion was caused by *Neospora* in Iran addressed rates of 13% for abortion (Razmi et al, 2007) and 14.4% for total vertical transmission (Razmi et al, 2010), which was almost similar to the findings of this research.

According to other investigations, the rate of vertical transmission in cattle varies from 4 to even 100 percent and the rate of abortion in these studies is around 11%; the rate of abortion in animals with a high titre of anti-*Neospora* antibody was usually shown to be higher than that in animals with no titre of the antibody (Dubey et al, 2007; Macedo et al, 2013). In our results, statistical analysis showed that the relative risk for the vertical transmission and the rate of transplacental transmission for *N.caninum* from seropositive mothers to their ABH calves were 31.2 and 42.85%, respectively (Table 2). The high relatively risk for transplacental transmission of the parasite emphasizes that the vertical transmission plays an important role in the remaining and distribution of the infection in large dairy farms.

All of the serum samples in this study were tested for *Neospora* DNA by PCR and further confirmed by nested-PCR. The results showed that the *Neospora* DNA was only detectable in the sera containing anti-*Neospora* antibody with OD above the cut-off point (OD more than 0.5). Similar results were also obtained in buffalo, where *Neospora* DNA was only detected in the sera of seropositive buffaloes (unpublished data). Although PCR positive results were observed merely in mothers and newborns with ELISA titers above the cut point, another interesting point of the present study is that, despite the high titer of the ELISA of 14 dams, 6 of them (42.85%) were unable to prevent placental transmission of the infection in contrast to 8 dams (57.14%). It seems that the recent group of 8 cattle have been able to show effective placental immunity despite the evidenc of the infection. The significant high level of ELISA titer in 6 congenitally infected calves is quite conceivable (Table 3). The results showed an overall rate of 12.24% for *Neospora* infection in AHB calves. All of the infected calves were born from seropositive mothers. These results confirmed the previous findings showing that the rate of vertical transmission of *Neospora* in seropositive cows was higher than that in seronegative cases (Santos et al, 2012).

Yet in naturally infected cattle, *Neospora* DNA has been detected in the semen, blood, brain and fetal tissues (Ferre et al, 2005; Okeoma et al, 2004). Detection of *Neospora* DNA in the serum of pregnant cows was also reported (McInnes et al, 2006). On the other hand, some previous studies addressed a high level of anti-*Neospora* antibody in acute phases of the infection and detection of *Neospora* DNA in the blood after parasitemia (Ferre et al, 2005;

Okeoma et al, 2005). Simultaneous detection of anti-*Neospora* antibody in the serum and *Neospora* DNA in WBCs was also reported in some studies (Okeoma et al, 2005; Okeoma et al, 2004); but this is the first report on a 100 percent concurrency in detection of *Neospora* DNA and anti - *Neospora* antibody in the serum of both mothers and their precolostral calves in cattle immediately after delivery. DNA of other protozoa such as *Toxoplasma*, which are highly similar to *Neospora*, was also detected by other researchers in mouse and human sera (Hafid et al, 2000; Meganathan et al, 2010). They believed that using the serum for diagnosis of such parasites by PCR, can be employed in retrospective studies (McInnes et al, 2006). Some other researchers could not find the *Neospora* DNA in the serum of seropositive cows with abortion caused by *Neospora,* so emphasized the lack of association between the presence of *Neospora* DNA and anti-*Neospora* antibodies in the serum (McInnes et al, 2006). Therefore, the association between the presence of DNA and anti-*Neospora* antibodies in *Neospora* infected cows occurs only when they are infected in the second semester of the pregnancy resulting in delivering AHB calves. In addition, we used a whole-cell based ELISA and it is thought that using the whole parasite renders a higher specificity for ELISA in diagnosing *Neospora*. In our results, the level of the antibody in 9 *Neospora* DNA negative cows was significantly high but below the cut-off point. It seems when the level of the antibody was higher than the cut-off point, the *Neospora* DNA was detectable in the serum and after removing the parasite from the blood by the immune system, the level of antibody in the serum dropped below the cut-off point.

Although in epitheliochorial placenta, bloods of mothers and foetuses are not mixed together (Dubey et al, 2006), the parasite is able to break the placental barrier in some of *Neospora* positive mothers and arrive the foetal blood producing a high level of the antibody in the serum of AHB calves before receiving the colostrum. Therefore, the antibody detected in the calves' sera was produced by the foetus itself. This confirms the capability of foetal immune system in producing an immune response against the parasite (Table 3). This was in line with some other studies, which reported a high level of anti-*Neospora* antibody in the sera of precolostral calves delivered by mothers with a history of *Neospora* caused abortion (Bou et al, 1999). It is known that pathogenic agents are recognized by the foetal immune system after the first trimester of pregnancy, so that the possibility of creating persistent infection by *Neospora* has been discussed by some of *Neospora* researchers (McInnes et al, 2006). So, the AHB calves of our study received the infection after the first trimester of pregnancy and the foetal immune system was able to recognize the parasite and prevent the abortion by producing the antibody.

In the present study, the similarity of ELISA and PCR results in mothers, was also observed in their AHB calves indicating a similar phenomenon occurred in both mothers and the calves.

There was a strong association between the presence of *Neospora* DNA and high levels of anti- *Neospora* antibody in the serum of *Neospora* infected cows and their precolostral AHB calves. Although placental immunity plays an effective role in preventing vertical transmission, this parasite can be transmitted vertically to calves born from infected mothers at a relatively high rate. The placental barrier is capable to stop the parasite from transmission and needs to be more investigated.

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Conflict of interest

We declare that there is no conflict of interest.

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References

Bou, G., Figueroa, M. S., Marti-Belda, P., Navas, E., & Guerrero, A. (1999). Value of PCR for detection of *Toxoplasma gondii* in aqueous humor and blood samples from immunocompetent patients with ocular toxoplasmosis. *Journal of Clinical Microbiology*, *37*(11), 3465-3468.

Dubey, J. P. (2003). Review of *Neospora caninum* and neosporosis in animals. *Korean Journal of Parasitology*, 41(1), 1-16.

Dubey, J. P., Buxton, D., & Wouda, W. (2006). Pathogenesis of bovine neosporosis. *Journal of Comparative Pathology*, 134(4), 267-289.

Dubey, J. P., & Schares, G. (2006). Diagnosis of bovine neosporosis. *Veterinary Parasitology*, 140(1-2), 1-34.

Dubey, J. P., Schares, G., & Ortega-Mora, L. M. (2007). Epidemiology and control of neosporosis and *Neospora caninum*. *Clinical Microbiology Reviews*, 20(2), 323-367.

Ferre, I., Aduriz, G., Del-Pozo, I., Regidor-Cerrillo, J., Atxaerandio, R., Collantes-Fernandez, E., Ortega-Mora, L. M. (2005). Detection of *Neospora caninum* in the semen and blood of naturally infected bulls. *Theriogenology*, 63(5), 1504-1518.

Guy, CS, Williams, Diana JL, Kelly, DF, McGarry, JW, Guy, F, Björkman, C, Trees, Alexander J. (2001). *Neospora caninum* in persistently infected, pregnant cows: spontaneous transplacental infection is associated with an acute increase in maternal antibody. *The Veterinary record*, 149(15), 443-449.

Hafid, J., Guichard, D., Flori, P., Bourlet, T., Raberin, H., Genin, C., & Sung, R. T. (2000). Detection of *Toxoplasma gondii* by polymerase chain reaction in sera of acutely infected mice. *Journal of Parasitology*, 86(4), 857-859.

Hajikolaei, Haji M. R., Goraninejad, S., Hamidinejat, H., , Ghorbanpour, M., & Paryab, R. (2007). Occurrence of *Neospora caninum* antibodies in water buffaloes (Bubalus bubalis) from the south-western region of Iran. *Bulletin of the Veterinary Institute in Pulawy*, *51*(2), 233.

Macedo, C. A., Macedo, M. F., Cardim, S. T., Paiva, M. C., Taroda, A., Barros, L. D., Garcia, J. L. (2013). *Neospora caninum*: evaluation of vertical transmission in slaughtered dairy cows (Bos taurus). *Revista Brasileira de Parasitologia Veterinaria*, 22(1), 13-17.

McInnes, L. M., Ryan, U. M., O'Handley, R., Sager, H., Forshaw, D., & Palmer, D. G. (2006). Diagnostic significance of *Neospora caninum* DNA detected by PCR in cattle serum. *Veterinary Parasitology*, 142(3-4), 207-213.

Meganathan, P., Singh, S., Ling, L. Y., Singh, J., Subrayan, V., & Nissapatorn, V. (2010). Detection of *Toxoplasma gondii* DNA by PCR following microwave treatment of serum and whole blood. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 41(2), 265-273.

Nasir, A., Lanyon, S. R., Schares, G., Anderson, M. L., & Reichel, M. P. (2012). Sero-prevalence of *Neospora caninum* and *Besnoitia besnoiti* in South Australian beef and dairy cattle. *Veterinary Parasitology*, 186(3-4), 480-485.

Okeoma, C. M., Stowell, K. M., Williamson, N. B., & Pomroy, W. E. (2005). *Neospora caninum*: quantification of DNA in the blood of naturally infected aborted and pregnant cows using real-time PCR. *Experimental Parasitology*, 110(1), 48-55.

Okeoma, C. M., Williamson, N. B., Pomroy, W. E., Stowell, K. M., & Gillespie, L. (2004). The use of PCR to detect *Neospora caninum* DNA in the blood of naturally infected cows. *Veterinary Parasitology*, 122(4), 307-315.

Pare, Julie, Thurmond, Mark C, & Hietala, Sharon K. (1997). *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. *The Journal of parasitology*, 82-87.

Razmi, G. R., Maleki, M., Farzaneh, N., Talebkhan Garoussi, M., & Fallah, A. H. (2007). First report of *Neospora caninum*-associated bovine abortion in Mashhad area, Iran. *Parasitology Research*, 100(4), 755-757.

Razmi, G. R., Zarea, H., & Naseri, Z. (2010). A survey of *Neospora caninum*-associated bovine abortion in large dairy farms of Mashhad, Iran. Parasitology Research, 106(6), 1419-1423.

Santos, R. R., da Rocha, C. M., Goncalves Tde, M., & Guimaraes, A. M. (2012). Quantification of vertical transmission of *Neospora caninum* in dairy cows in Minas Gerais, Brazil. *Revista Brasileira de Parasitologia Veterinaria*, 21(3), 294-297.

Stenlund, Susanne, Kindahl, Hans, Magnusson, Ulf, Uggla, Arvid, & Björkman, Camilla. (1999). Serum antibody profile and reproductive performance during two consecutive pregnancies of cows naturally infected with *Neospora caninum*. *Veterinary parasitology*, 85(4), 227-234.

Yu, Jinhai, Xia, Zhaofei, Liu, Qun, Liu, Jing, Ding, Jun, & Zhang, Wei. (2007). Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes (Bubalus bubalis) in the People's Republic of China. *Veterinary parasitology*, *143*(1), 79-85.

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ارتباط بین حضور DNA و آنتی‌بادی سرم در طول انتقال عمودی *نئوسپورا کنیوم*

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چکیده

نئوسپورا کنیوم یک انگل درون سلولی است که باعث سقط جنین و نارسایی تولید مثل در گاو می‌شود. هدف از مطالعه حاضر، ارتباط بین عفونت انگلی و مثبت شدن سرم در گاوهای بدون علامت سقط و گوساله‌های آن‌ها، بود. جهت این بررسی، ۴۹ نمونه سرم از گاوهای شیری با ظاهر سالم و گوساله‌های تازه متولد شده قبل از دریافت آغوز توسط Nested-PCR,PCR و یک روش الایزا مبتنی بر سلول کامل آزمایش شد. ۱۴ مورد از ۴۹ مادر (۲۸/۵۷ درصد) و ۶ مورد از ۴۹ گوساله (۱۲/۲۴ درصد) آنتی‌بادی ضد *نئوسپورا* و DNA *نئوسپورا* را در سرم نشان دادند. بر اساس آنالیزهای مختلف سرمی، همه گوساله‌های آلوده از مادران آلوده به دنیا آمدند و میزان انتقال عمودی در بین همه نمونه‌ها ۶ مورد از ۴۹ مورد (۱۲/۲۴ درصد) و از مادران آلوده ۶ مورد از ۱۴ مورد (۴۲/۸۴ درصد) بود، بر اساس آنالیزهای مختلف سرمی، هشت گوساله از ۱۴ گوساله (۵۷/۱۴ درصد) متولد شده از مادران آلوده عاری از *نئوسپورا* بودند. در پایان، علی‌رغم وجود آنتی‌بادی‌های ضد *نئوسپورا* سرم در گاوها و گوساله‌های آلوده، انگل به طور کامل از خون خارج نشد و به نظر می‌رسد وجود آنتی‌بادی لزوماً نشانه‌ای از ایمنی موثر نیست.

کلمات کلیدی: *نئوسپورا کنیوم*، آنتی‌بادی، DNA

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