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Original Article

Neospora caninum - Associated Abortions in Slovak Dairy Farm

***Silvia ŠPILOVSKÁ, Katarína REITEROVÁ, Daniela ANTOLOVÁ**

Institute of Parasitology of the Slovak Academy of Sciences, Košice, Slovak Republic

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***Correspondence**
Email:
spilovska@saske.sk

Abstract

Background: *Neospora caninum* is considered one of the major causes of repeated abortions in livestock. This study aimed to determine the seropositivity to *N. caninum* using indirect ELISA and the influence of the infection on the occurrence of abortions in selected dairy herd in Slovakia.

Method: Blood samples were obtained from 490 cattle over a period of two years and were tested for *N. caninum* antibodies using indirect ELISA.

Results: The presence of specific antibodies in the herd was detected in 118 (24.1%) cows. According to selected groups; 117 (41.0%) cows with a history of abortion, 65 (43.3%) heifers and 223 (2.2%) cows without abortions were tested positive to *Neospora*. Vertical transmission of *N. caninum* dominated in examined herd and the relative risk (RR) of dam-daughter seropositivity in progenies of seropositive mothers was 2.1 times higher than in progenies of seronegative dams. Molecular analyses of aborted foetuses of seropositive mothers showed the presence of *Neospora* DNA. However, 23 (28.1%) of heifers born to seronegative cows were seropositive, indicating also the postnatal transmission of the infection from the environment.

Conclusion: Study revealed significant correlation between the presence of specific antibodies and the occurrence of abortions, the risk of abortion in seropositive animals was 3.8 times higher than in seronegative ones. Incorrect farm management contributed to spread and circulation of neosporosis in entire dairy herd what could significantly impair the reproduction and economic parameters of breeding.

Introduction

Intracellular protozoan parasite, *Neospora caninum,* is distributed worldwide and is considered one of the major causes of repeated abortions in livestock (1). Dogs (*Canis lupus familiaris*) were the first discovered definitive hosts of this parasite. Other natural

definitive hosts are coyotes (*C. latrans*), dingo (*C. lupus dingo*) and grey wolves (*C. lupus lupus*) (2). The definitive hosts transiently shed oocysts after ingestion of *N. caninum*-infected body tissues from intermediate hosts, contaminate the environment and serve as a source of the infection (3). In the life cycle of *N. caninum*, both horizontal and vertical transmission of parasite has been reported. However, the dominant way of infection is vertical transmission. Cows may remain infected with *N. caninum* for their whole life and transmit the infection over several generations (4).

Infection of the embryonic environment can be caused by specific (e.g. *T. gondii, N. caninum, Campylobacter foetus,* BVDV, BHV-*1)* and non-specific *Brucella abortus*, *Arcanobacter pyogenes*, *Candida* spp., *Leptospira* spp., *Listeria* spp., *Haemophilus somnus* etc.) pathogens (5).The first serological study in Slovakia revealed 20% mean seroprevalence of *N. caninum* in dairy cattle post-abortion while in cows without any reproduction problems it reached only 2.3% (6).

The aim of the present study was to determine the positivity to *Neospora caninum* and the influence of the infection on the occurrence of abortions in selected dairy herd in Slovakia. The molecular analyses of aborted foetuses in the herd were also performed.

Material and Methods

Characteristics of dairy herd

The study focused on a large dairy farm with a high incidence of abortions. Dairy farm breeding Slovak spotted breed (75%), Ayrshire (25%) and their crossbreeds is situated in the northeastern part of Slovakia. The region is characterised by broad-leaved forests and a great biological diversity of animals with a high abundance of deer and wild carnivores.

The herd of 590 cattle consisted of 50 calves (from new-born to 4 months of age), 150 heifers (6–20 months), 50 bulls (4–24 months) and 340 cows (>20 months). Bulls and calves were not serologically tested due to the owner decision. Mean milk production in the herd was 4500 kg milk per lactation. Semi-extensive farming was used in dairy herd management. Cattle were in free-stall housing and divided according to the stage of reproduction cycle and milk production. In the group of 340 cows, 117 had a history of abortion (abortion in dairy cattle is defined as a loss of the foetus between the age of 42 days and approximately 260 days of pregnancy).

Serological evaluation

Blood samples were obtained from the entire herd of 490 cattle over a period of two years. Sera were separated and stored at −20 °C until tested for *N. caninum* antibodies. An indirect ELISA (ID-VET Company, France) was used to evaluate sera for *N. caninum* antibodies. The absorbance (OD) was measured at 450 nm by spectrophotometer (Thermolabsystem, Opsys MR, U.S.A.). For each sample, the ratio of optical density of examined serum to mean OD of positive controls was calculated as S/P%. Samples with the S/P% ≤ 50 were classified as negative and the samples with S/P% > 50 were considered positive.

Aborted foetuses

During the survey of neosporosis, four entire aborted foetuses from the farm (fifth to sixth month of gestation) were transported in cold conditions to the laboratory immediately after the abortion. Approximately 5 g of brain tissue of each foetus was homogenized in 20 ml of sterile PBS (phosphate saline buffer), transferred to microtubes, and then stored at -20 °C until molecular analysis. During the necropsy blood samples from the heart were also collected, centrifuged and serum samples were stored at -20 °C. Blood samples of dams were collected and serologically tested, too. Due to the high degree of autolysis of foetuses the histopathology was not done.

Molecular analyses

All brain tissue samples were homogenised and digested by 0.1% trypsin for one hour at the temperature of 37 °C. Genomic DNA was isolated from the pre-digested tissue sediments using the Nucleospin® Tissue DNA extraction kit (Machery-Nagel, Germany). PCR was performed using the primer pair Np21− Np6 (7). The PCR reactions were performed in a 25 µl volume containing 30 pmol of each primer, 1.25 U of *Taq* DNA polymerase, 10x PCR buffer, 200 µM of each deoxynucleoside triphosphate, 1 µl of DNA and sterile ddH_2O. The amplification was carried out in the thermocycler (Bioer, China) under the following conditions: initial denaturation of templates at 95 °C for 7 min, followed by 35 cycles of denaturation (95 °C, 30 sec.), annealing (55 °C, 30 sec.) and extension (72 °C, 1 min), with a final extension at 72 °C for 10 min. PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide, in a running buffer of 0.5% TBE at 75 V for 1 hour. As positive and negative controls, *N. caninum* tachyzoites (first Slovak isolate NC-SKB1) (8) cultured on Vero cells and uninfected Vero cells were used.

Statistical analyses

The results were analysed statistically using Fisher's exact test. The prevalence values were given with 95% confidence interval (CI) for proportions. Relative risk (RR – the ratio of the probability of an event occurring in an exposed group to the probability of the event occurring in a comparison, non-exposed group) and odds ratio (OR – an expression of the quantity of one substance or entity in relation to that of another) were also calculated. Statistical analyses were conducted using Statistica 6.0 (Stat Soft, Tulsa, USA) and a value of $P < 0.05$ was considered significant.

Results

Seropositivity to N. caninum

Results of whole-herd screening revealed 118 cows (24.1%) seropositive to *N. caninum*. In cows with history of abortion 48 (41.0%) in cows without abortions 5 (2.2%) and in heifers 65 (43.3%) had antibodies against *N. caninum*.

The occurrence of anti-*Neospora* antibodies among heifers born to *N. caninum* seropositive cows (61.7%) was significantly higher ($P = 0.005$) than in heifers born to seronegative mothers (28.1%). In offspring of seronegative mothers the number of seronegative heifers (72.2%) significantly prevail ($P = 0.0005$) the number of seropositive ones. According to analyses, the relative risk of seropositivity in progenies of seropositive mothers was 2.1 times higher than in progenies of seronegative dams. Totally, 28.1% of heifers born to seronegative cows were seropositive to *N. caninum*, suggesting the occurrence of postnatal infection (horizontal transmission) in the herd (Table 1).

Table 1: The occurrence of anti-*Neospora* antibodies in heifers born from *Neospora* seropositive or seronegative cows

HEIFERS	COWS	
	Seropositive	Seronegative
	No (%)	
Seropositive	42 (61.7)	23 (28.1)
Seronegative	26 (38.2)	59 (72.2)
Odds ratio= 4.1	Relative risk= 2.1	$P<0.005$

Incidence of abortions in seropositive and seronegative cows

Significant differences ($P < 0.0001$) in the occurrence of abortions in relation to seropositivity to *Neospora* were detected. As much as 90.6% of seropositive dams had positive history of abortion, while only 5 (9.4 %) did not abort previously. Thus, relative risk of abortion was 3.8 higher in seropositive cows then in animals without antibodies to *N. caninum*. Similarly, very high OR (30.3) suggests significant association between seropositivity and occurrence of abortions (Table 2).

Table 2: The occurrence of abortions in seropositive and seronegative cows

COWS	After abortion No. (%)	Without abortion No. (%)	95% CI – confidence interval
Seropositive	48/90.6	5/9.4	0.121-0.198
Seronegative	69/24.0	218/76.0	0.802-0.879
Odds ratio= 30.3	Relative risk= 3.8	$P<0.0001$	

Serology and molecular detection of Neospora caninum in aborted foetuses

Serological analyses confirmed dam-daughter seropositivity as anti-*Neospora* antibodies were found in three out of four serum samples of aborted foetuses (S/P% 78.3%, 64.9% and 72.4%) and their mothers (S/P% 187.5, 176.6% and 192.3%). The polymerase chain reaction amplified the specific amplicons of 328 bp in brains of all three foetuses aborted by seropositive cows In brain tissue of a foetus aborted by seronegative dam no *N. caninum* DNA was found, what is in accordance with their negative serology.

Discussion

Neosporosis has aroused an immense interest mainly due to repeated abortions in cows and a negative effect on their breeding economy. The majority of *N. caninum* infections appear to have a chronic course, probably causing life-long persistence of the parasite in tissues of infected animals (1).

In this study, serological screening confirmed the influence of *N. caninum* on the occurrence of abortions in selected dairy herd. The whole herd screening revealed 24.1% seropositivity to *N. caninum*. The occurrence of anti-*Neospora* antibodies was by 38.8% higher in a group of cows with history of abortion when compared to cows with no record of abortion.

The predominant route of *N. caninum* transmission in the herd was analysed by comparison of dam-daughter serostatus. The occurrence of anti-*Neospora* antibodies among heifers born to *N. caninum* seropositive cows was significantly higher than in heifers born to seronegative mothers, suggesting the dominance of transplacental (vertical) transmission of *N. caninum* in the herd. According to statistical analyses, seropositivity of dams increased the risk of infection of their progenies 2.1 times.

The vertical mode of the disease transmission confirmed also the finding of *N. caninum* DNA in brains of all three foetuses aborted by seropositive dams. During the acute phase of the disease, tachyzoites can be found in several organs of intermediate hosts. However, the brain is the most frequently affected organ, with the most protozoan tissue cysts observed in both, the acute and chronic phase of the infection (9). Ortega-Mora et al. (10) reported that high probability of vertical transmission correlates with the seropositivity in different age groups of animals and with serological status of dams and their offspring. Some authors also recorded very high rate of congenital transmission in seropositive cows, 81% and 94%, respectively (11, 12). In other studies, 67.53% (13) and 36.8% (14) positive serology was found in foetuses from *N. caninum* seropositive cows. Thus, *Neospora*-infected cows present severe risk of transplacental transmission of parasite to their progenies.

In endemic conditions, cattle can become also infected by consuming feed or water contaminated by oocysts or grazing on contaminated pastures. In 28.1% of positive cases, no association between the serological status of dams and daughters was observed, suggesting the existence of horizontal transmission of the parasite. French et al. (12) concluded that *N. caninum* infection cannot be sustained in herds without horizontal transmission. On the farm, the presence of dogs with free access to sta-

bles and pastures increases the risk of environmental contamination by parasite oocysts. Moreover, the farm borders with the National Park of Poloniny characterised by high population density of wild carnivores. The results also confirmed that in bovine herd the positivity to *N. caninum* is in correlation with impaired female reproduction. Abortion as one of the most important symptoms of seropositivity in cattle may occur due to activation of tachyzoites and their migration to the uterus. It has been accepted that cattle exposed to the parasite for the first time are primarily at risk of abortion and the aborting cow usually has no other clinical signs (15). We confirmed the significant correlation between the seropositivity and incidence of abortions; as more than 90% of seropositive dams had positive history of abortion and relative risk of abortion was 3.8 higher in seropositive cows then in animals without antibodies to *N. caninum*. Corbellini et al. (16) recorded that *N. caninum* seropositive dams have 3.3 times higher risk of abortion than seronegative ones. In the other study, congenitally infected cows had a 7.4-fold higher risk of abortion during their initial pregnancy compared with noninfected cows (17).

The association between positive serology for *N. caninum* and abortion during the reproductive period, when compared to negative cows, may confirm the presence of the disease in the herd. Recently, Hamidimejat et al. (18) performed easy application of DOT–ELISA to detect anti-*Neospora* antibodies what may be beneficial also in condition when the laboratory equipment is not available. The positive serology suggests both, the existence of antibody production against the tachyzoites (active stage) and at least the presence of bradyzoites, which can remain viable for the rest of the host life (latent stage).

Conclusions

The study confirmed correlation between the high incidence of anti-*Neospora* antibodies and occurrence of abortions in monitored farm. The vertical mode of the infection transmission dominated and contributed to maintenance of *N. caninum* in the herd over several following bovine generations. On the other hand, the horizontal transmission of the infection was also detected. We can conclude that incorrect farm management contributed to the spread and circulation of neosporosis in entire dairy herd, what significantly impaired the reproduction and economic parameters of breeding. On that account, bovine neosporosis should be controlled regularly by serological monitoring of cows, and examination of aborted foetuses, eventually by precolostral serology of calves is recommended. The results emphasize the need to implement prevention programme in strategies for culling of seropositive females in dairy herd.

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