

Reproductive Problems Due to *Neospora Caninum* in Dairy Cows from Greece

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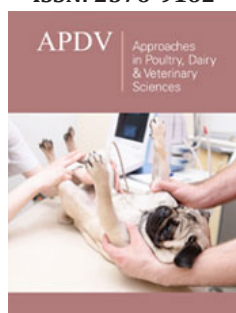
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ISSN: 2576-9162



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Submission:  January 20, 2022

Published:  January 31, 2022

Volume 8 - Issue 5

How to cite this article: Menelaos Lefkaditis, Zoi Athanasakopoulou, Rustem Bayramoglou, Marina Sofia, Dimitris C Chatzopoulos, Vassiliki Spyrou and Charalambos Billinis. Reproductive Problems Due to *Neospora Caninum* in Dairy Cows from Greece. *Appro Poultr Dairy & Vet Sci* 8(5). APDV. 000700. 2022. DOI: [10.31031/APDV.2022.08.000700](https://doi.org/10.31031/APDV.2022.08.000700)

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Abstract

Neospora caninum is a major cause of reproductive problems in cattle worldwide. The aim of the present study was to describe the rate of *N. caninum* infection among cows that presented reproductive problems in Greece. Nine hundred and thirty-one blood samples were collected from an equal number of dairy cows that had presented abortion or decreased reproductive performance and were subjected to serological detection of IgG *N. caninum* antibodies. Subsequently, brain tissue samples from aborted fetuses were collected from cows that were found positive for *N. caninum* antibodies and were molecularly tested for the presence of the parasite. Antibodies against *N. caninum* were found in 200 of the blood samples tested and the presence of the parasite was genomically confirmed in all the examined fetal brain tissue samples. This report provides evidence of *N. caninum* role as a cause of cattle reproduction problems in Greece and underlines the need for the implementation of effective preventive measures.

Keywords: *Neospora caninum*; Neosporosis; Cattle; Reproductive problems; ELISA

Introduction

Neospora caninum (Sarcocystidae) is a protozoan parasite which is the etiological agent of neosporosis [1-3]. This apicomplexan parasite was initially recognized in 1984 from dogs in Norway [4] and was described as a new genus and species in 1988 [5]. In the *N. caninum* life cycle, dogs and other related canids are, beside their role as intermediate hosts, the only definitive hosts that shed through their feces the oocysts for a variable period of time into the environment [6-9]. *N. caninum* has been reported in a large number of intermediate hosts worldwide, such as ruminants, horses, rabbits, mice, deer, badgers, polecats, ferrets and mink [10-12], while it is not considered as a zoonotic agent although antibodies against *N. caninum* have been reported in human tissues [13]. Neosporosis has emerged as a serious disease only in cattle and dogs. Regarding cattle, neosporosis is recognized as one of the most important causes of cow reproductive and infertility problems and abortions worldwide while it can also cause increased mortality in newborn calves [8,10]. When a cow becomes infected from dog feces, the infection spreads via tachyzoites to other tissues of the body and to the placenta in cases of pregnancy, where damage to the placenta or vertical transmission through the placenta to the fetus can occur. Vertical transmission of *N. caninum* is considered the principal route of infection in cattle [10] while a cow may pass the infection to multiple offspring [14]. Abortion may be a result of both the primary damage and the immune mediated inflammatory response of the cow [15,16]. Calves that are infected in utero may be born weak, underweight and with neurological symptoms such as ataxia, decreased reflexes and exophthalmia [3,8]. Neosporosis has a serious economic impact within a flock especially if the prevalence is high. This results from direct costs such as the value of fetuses and indirect costs, including veterinarian support, costs associated with rebreeding, possible loss of milk yield, and replacement costs if aborted cows are culled [17,18]. The aim of this study was to describe the rate of *N. caninum* infection among cows that presented reproductive problems in Greece.

Materials and Methods

During 2018-2021, blood samples were collected in Greece from 931 dairy cows that presented reproductive problems, including abortions (n=384) and decreased reproductive performance (n=547). All samples were examined by an indirect Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of IgG antibodies against *N. caninum* (IDVet, Montpellier, France). In addition, tissue samples from aborted fetuses were collected from cows that were found positive for *N. caninum* antibodies and were examined by nested polymerase chain reaction (PCR) for *N. caninum*. The presence of the NC-5 gene was evaluated using the primers and conditions described by Corbellini [19].

Results

Antibodies against *N. caninum* were found in 200 (21.5%) of the 931 samples tested (Table 1). Eighty-seven of the positive samples originated from cows that had aborted and 113 from cows with decreased fertility. Among the cows that presented abortion, 48 had aborted in the first lactating period, 10 in the second and 29 in early gestation (32-70 days). Nested PCR for *N. caninum* in seven brain samples, which were collected from the aborted fetuses of an equal number of positive for *N. caninum* cows, recorded a positive result. Brain samples were not collected from the fetuses of all the cows that had aborted either due to early abortion (32-70 days) and subsequent insufficient development of the brain (n=29) or due to advanced autolysis of the fetus (n=51).

Table 1: Characteristics of the farms included in the study.

Farm	Total Number of Animals in the Farm	Number of Sampled Animals with Reproductive Problems	Animals Positive for <i>N. Caninum</i> Antibodies	Animals Negative for <i>N. Caninum</i> Antibodies
F1	2100	100	9	91
F2	1431	231	52	179
F3	165	90	14	76
F4	217	110	18	92
F5	955	215	75	140
F6	457	98	23	75
F7	243	87	9	78
Total	5568	931	200	731

Discussion

Abortions and neonatal mortality are a major problem in livestock. Neosporosis is recognized as one of the most important causes of reproductive problems and abortion in cattle worldwide [8,10,20,21]. Abortions and neonatal mortality can cause severe financial losses, especially when the disease is endemic or epidemic. In this study a large amount of reproductive problem cases was investigated using the combination of two techniques (ELISA and PCR). The results recorded in our research showed the spread of Neosporosis among 21.5% of the dairy cows that had such problems. Previous studies have shown prevalence of *N. caninum* infection among cows presenting abortion that varies from 12 to 42% [22-25]. Abortions due to *N. caninum* have been described to occur starting in month three of gestation and until delivery [10,21,26] in an epidemic or endemic manner [27]. Of note, our study records several cases occurring between the first 32 to 70 days of gestation. In addition, the present study records that most miscarriages due to neosporosis occurred during the first lactation period, which indicates that the infections were recent and that preventive measures should be implemented on the infected farms. *N. caninum* can also cause fetal viability disorders or neurological birth defects in newborn calves [28,29] and calves younger than 2 months of age [30]. The *N. caninum*-infected young calves may present neurologic signs, low birth weight [6], difficulties to rise and stand, flexed or hyperextended hind and/or forelimbs, and in some cases exophthalmia or asymmetrical appearance of the

eyes. However, most of the calves born congenitally infected remain clinically healthy [31-33]. The aforementioned situations lead to additional financial losses for the breeders and, thus, the employment of preventive measures is necessary [32]. In cattle, the transplacental transmission is the most frequent route of infection, being observed in up to 93.7% of cases [10]. Dogs play an important role in the horizontal transmission and maintenance of *N. caninum* infection in dairy cattle [6-8] and subsequently keeping dogs away from infected breeding is recommended [34].

Conclusion

Neosporosis is a very important parasitic disease which can cause infertility, abortions, neonatal mortality and other clinical symptoms in dairy cattle. Frequent preventive laboratory tests are needed to determine the causes of infertility, miscarriages and birth of calves with characteristic clinic symptoms. Preventive measures and special management are needed in infected flocks with *N. caninum*.

Acknowledgment

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (Acronym: the summer cow, project code: T1EDK-01078).

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