

NEOSPORA CANINUM ANTIBODIES IN BULK MILK AND SERUM FROM ESTONIAN DAIRY FARMS

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ABSTRACT

Neospora caninum is a protozoan pathogen related to abortions and stillbirths of cattle around the world. We present the first results in Estonia on this parasite. For analysis of bulk milk and serum samples SVANOVIR@Neospora-ab ELISA kit (SVANOVA) were used with cut-off $\geq 20\%$ of the positive control (percent of positive value, PP). Bulk milk samples from 2007 and 2008 (N=65 and N=320) were screened, resulting in 26 and 16% herd prevalences. Additionally, two farms positive in the bulk milk testing were selected for serological examination of individual cattle (N=59 and N=20). Serum were positive for Neospora in 23% of the tested animals. Estonia has considerable levels of antibody positive herds and animals, and Neospora is likely to be a cause of abortions. Future case studies are required to verify Neospora as cause of abortions.

KEYWORDS: Neospora caninum, cattle, Estonia, milk

INTRODUCTION

Neospora caninum has long been accepted to be a cause of abortion, stillbirths, and deformations of calves [1]. Though congenital infections is important (vertical transmission) probably is most common, postnatal infections, most likely from the definitive host (the dog), is probably required to keep the infection in the herd [2]. In Estonia udder diseases and reproductive problems are competing to be the leading cause of culling in dairy production animals, but investigations of Neospora has not previously been attempted [6]. In order to establish an estimate of farms with *Neospora caninum* we screened bulk milk and serum samples for specific antibodies.

MATERIALS AND METHODS

Bulk milk samples from 65 and 320 different dairy farms were collected in 2007 and 2008 respectively, and tested for antibodies against Neospora using and following the instructions of the commercially available iscom-ELISA kit (SVANOVIR® Neospora-ab ELISA, SVANOVA). Skimmed milk samples were diluted 1:2 and serum 1:100 in PBS-tween. Cut off value $\geq 20\%$ of the kits positive control (PP = OD sample/OD positive control * 100), as recommended by the manufacturer. Two positive herds from the 2007 study were selected and 59 and 24 different blood samples were tested respectively for Neospora antibodies with the same kit.

RESULTS AND DISCUSSION

Samples collected in 2007 tested 17 out of 65 herds (26%) positive for Neospora. The two farms examined for serum antigens had 20 (33%) and 5 (19%) animals positive respectively. Average of the individual samples was calculated to 23%. Fifty two bulk milk samples out of 320 herds (16%) were positive for the parasite in 2008 (Table 1).

Although the used test is good at detecting *Neospora caninum* in infected herds, it is difficult to establish more than the presence of the pathogen in a sample. In this study it is clear that around 1/5 Estonian herds have infected animals. More serum samples needs examination to establish an individual prevalence, with inclusion of farms testing negative on the bulk milk

sample. The tested serum from the two positive farms gives the impression that bulk milk positive farms can have a fairly large number of animals positive as well in Estonia. When comparing to other studies investigating *Neospora caninum* prevalences by the presence of antibodies, care has to be taken due to the variety in methods. European studies report prevalences in individual cattle serum samples between 2-36%, with an average around 11% [2]. Our selected farms averaged 23% of all animals seropositive, but a larger sample size is needed for comparison. If only comparing to other studies with the same method examining bulk milk prevalences we found 1.01% in Czech (N=495), and 15.5% in Latvia herds (N=142) [2,3]. Compared to these studies Estonia seems to have more farms with infected animals. Presence of antibodies against *Neospora caninum* does not equal an aborting animal [5]. Risk of abortion in seropositive cow studies do show a dramatically 2-3,5 times increase, and up to 7,4 times in congenital infected heifers in first pregnancy [6,9]. There is still unsolved mysteries regarding the transmission of dog oocysts, and cattle infections. Studies do indicate there is a connection between seropositive cattle and the occurrence and amount of farm dogs [7,8]. Dogs are commonplace companion animals in Estonian farms. Interesting for further examinations of aborted calves and fetuses is of interest in establishing the extent of neosporosis as a causal factor in Estonia. Additionally, we plan to examine the serology of our tested samples in this study for additional pathogens to evaluate the risks factors associated with abortions.

Table 1

Herds and cattle tested for *Neospora caninum* antibodies

	N	Cut-off (% of positive control)	Range of positives (%)	Positive (N)	Positive (%)
2007					
Bulk milk	65	≥20	21-53	17	26
Blood, farm 1	59	≥20	20-108	20	33
Blood, farm 2	24	≥20	20-90	5	19
2008					
Bulk milk	320	≥20	20-106	52	16

CONCLUSION

Estonian herd and animal antibody prevalence can be considered a possible contributing factor to abortions and reproductive problems. Methods to investigate case studies to determine *Neospora* as an actual cause of reproductive problems is being implemented. The data is also currently being used in a wider epidemiological study of the risks involved in abortions.

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