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Universidade Federal de Santa Maria  
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Romero Nicolino, Rafael; Oliveira Capanema, Renato; Fonseca de Oliveira, Camila  
Stefanie; Oviedo Pastrana, Misael Enrique; Bastos Lopes, Luciano; Amaral Haddad,  
João Paulo

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## Estimating the abortion risk difference in *Neospora caninum* seropositive dairy cattle in Brazil

### Estimativa da diferença do risco de aborto em vacas leiteiras soropositivas para *Neospora caninum* no Brasil

Rafael Romero Nicolino<sup>I</sup> Renato Oliveira Capanema<sup>I</sup> Camila Stefanie Fonseca de Oliveira<sup>I</sup>  
Misael Enrique Oviedo Pastrana<sup>I</sup> Luciano Bastos Lopes<sup>II</sup> João Paulo Amaral Haddad<sup>\*</sup>

#### ABSTRACT

*Neosporosis in cattle herds is associated with large economic losses, with abortion being the only clinical sign perceptible to the producer. Losses are estimated at over one billion dollars worldwide. This study aimed to estimate the abortion risk difference in seropositive animals using specific data for dairy herds in Brazil. Differences in the risk of abortion between seropositive and seronegative animals were calculated through a meta-analysis of previous data from several Brazilian states, and an increase of 10.04% (0.091 to 0.118) in the specific risk was identified. This finding indicates that more than 474,000 abortions caused by neosporosis may be occurring only in dairy cattle herds in Brazil, causing a major economic loss in the milk production chain. The use of this specific measure for Brazilian herds opens the possibility of developing cost-benefit analysis for neosporosis in Brazil using data that are more reliable.*

**Key words:** abortion risk, *Neospora caninum*, dairy herd, economic modeling, Brazil.

#### RESUMO

*A neosporose está relacionada a grandes perdas econômicas em rebanhos bovinos, tendo o abortamento como único sinal clínico perceptível ao produtor, com prejuízos estimados em mais de um bilhão de dólares em todo o mundo. O presente trabalho teve como objetivo estimar a diferença no risco de aborto em animais soropositivos, especificamente com dados procedentes de rebanhos leiteiros do Brasil. A diferença do risco de aborto foi calculada através de uma meta-análise, com dados precedentes de vários estados brasileiros, tendo como resultado um aumento de 10,04% (0,091-0,118) no risco específico. Este resultado demonstra que podem estar ocorrendo mais de 474 mil abortamentos no Brasil, devido à neosporose, apenas em rebanhos bovinos leiteiros, causando grandes perdas econômicas para cadeia produtora de leite. A utilização dessa medida específica de rebanhos brasileiros*

*abre a possibilidade de modelagem econômica do custo da doença no Brasil com dados mais fidedignos.*

**Palavras-chave:** risco de aborto, *Neospora caninum*, bovinocultura de leite, modelagem econômica, Brasil.

#### INTRODUCTION

The milk production chain in Brazil produced more than 33 billion liters of milk from 22 million dairy cows in 2012, which is the third largest world production after the United States and India, generating four million jobs (IBGE, 2012). Despite this significant result, the fewer use of technology on the national herds and the lack of knowledge in health management persists. As a result, the introduction of pathogens in herds and their transmission between different categories of animals often trigger economic losses related to low production and reproductive performance.

The *Neospora caninum* is a protozoan which has wild canids and dogs as their definitive hosts and a variety of domestic species as intermediate hosts (GONDIM et al., 2004; DUBEY et al., 2007; COSTA et al., 2008; KING et al., 2010). Since the initial report of its presence in dogs from Norway in 1984 (BJERKAS et al., 1984) and the isolation and description of a new genus and species of protozoans called *Neospora caninum* (DUBEY et al., 1988), neosporosis has emerged as one of the main diseases causing an economic impact on cattle worldwide (HADDAD et al., 2005; DUBEY et al., 2007; REICHEL et al., 2013).

<sup>I</sup>Departamento de Medicina Veterinária Preventiva (DMVP), Universidade Federal de Minas Gerais (UFMG), 31270-901, Belo Horizonte, MG, Brasil. E-mail: jphaddad01@globo.com. <sup>\*</sup>Corresponding author.

<sup>II</sup>EMBRAPA Agrossilvipastoril, Sinop, MT, Brasil.

The large economic losses related to neosporosis in dairy cattle are due to reproductive failure that follows infection, including abortion, retained placenta, return to estrus, and increased calving intervals. It is estimated that 20% of all abortions that occur in Brazil, the Netherlands, and the United States may be associated with neosporosis (DUBEY et al., 2007). Losses related only to abortion are estimated at over one billion dollars worldwide, and in dairy herds, this loss reaches 843 million USD, with an estimated loss of 53 million USD in Brazilian herds (REICHEL et al., 2013).

An understanding of differences in the risk of abortion due to infection by *Neospora caninum* in cows and the various effects of the disease, such as a decline in milk production, transmission rates, and premature culling, is of fundamental importance to model the cost associated with the disease. There is no estimate of this risk difference specific for Brazilian's dairy herds, and the use of odds ratios to estimate the risk may result in overestimation ( $Odds > 1$ ) of the incidence of the disease (ZHANG et al., 1998).

In a meta-analysis study conducted in Canada in 2005, the abortion risk difference between seropositive and seronegative cows was reported to be 18% (SD=0.1) (HADDAD et al., 2005). In Brazil, REICHEL et al. (2013) estimated the specific risk of abortion due to infection by *Neospora caninum* at 14.3% (95% CI, 0.6%-39.4%). Although the study had a global scope and the authors highlighted the unavailability of specific abortion data for Brazilian

herds and used the average global risk to estimate the cost of abortion in Brazilian herds, it arrived at a wide confidence interval and less reliable results.

Estimation of the economic impact of neosporosis is essential in the pursuit for better results for the milk supply chain in Brazil. For this analysis, it is indispensable to know and estimate the physical effects of the disease in dairy herds. Herein the study reported the findings of a meta-analysis and discussed differences in the risk of abortion between seropositive and seronegative animals, fulfilling the current need of this measure specifically for national herds and targeting future economic modeling.

## MATERIALS AND METHODS

The abortion risk difference between seropositive and seronegative animals for *Neospora caninum* was performed through a meta-analysis (Table 1). This risk difference is an essential factor in the analysis to estimate the abortions costs due the neosporosis. Meta-analysis is an appropriate statistical technique that combines results from different studies, thus producing a result with greater power to estimate the real effect of the factor under study, also known as meta-analytical estimate, using the estimated effect and standard error of each study (RODRIGUES & ZIEGELMANN, 2010).

The random effects model was used, combining studies with similar objectives but conducted using different methods. Because the

Table 1 - Meta-analysis of the risk difference associated with *Neospora caninum* seropositivity in Brazilian's dairy herds.

Data	State	Diagnostic methods	Risk difference associated with <i>Neospora caninum</i> seropositivity		
			Risk difference	Lower Limit	Upper Limit
LOCATELLI-DITTRICH et al., 2001	Paraná	ELISA	0.124	0.052	0.196
CORBELLINI et al., 2002	Rio Grande do Sul	IFA <sup>1</sup>	0.154	0.027	0.281
SARTOR et al., 2003	São Paulo	IFA <sup>1</sup>	0.098	0.045	0.150
RAYMUNDO et al., 2005	Rio Grande do Sul	ELISA	0.146	- 0.026	0.318
CORBELLINI et al., 2006	Rio Grande do Sul	IHC <sup>2</sup>	0.167	- 0.006	0.339
OSHIRO et al., 2007	Mato Grosso	IFA <sup>1</sup>	0.189	0.048	0.330
SHULZE, 2008	Goiás	ELISA	0.121	0.096	0.147
GALVÃO et al., 2011	Bahia	IFA <sup>1</sup>	0.093	0.036	0.150
MUNHOZ et al., 2011	São Paulo	IFA <sup>1</sup>	0.215	- 0.053	0.483
CARDOSO et al., 2012	São Paulo	IFA <sup>1</sup>	0.087	0.057	0.116
HEIN et al., 2012	Rio Grande do Sul	IFA <sup>1</sup>	0.091	0.065	0.117
KATAGIRI et al., 2013	Paraná	IFA <sup>1</sup>	0.202	0.047	0.358
Results			0.104	0.091	0.0118

1 - Indirect immunofluorescence assay; 2 - Immunohistochemistry.

studies belonged to different regions of Brazil and included different herds, races, management, and diagnostic methods, the random effects model was considered appropriate. This method considers variations within and between studies and the different effects of neosporosis in each study, linking them through a probability distribution and establishing a confidence interval for the risk difference (RODRIGUES & ZIEGELMANN, 2010).

The inclusion criteria for this meta-analysis was: data from Brazilian dairy herds and the availability of sufficient data for the construction of tables to calculate the abortion risk difference, including information on the number of seronegative and seropositive animals and their historical abortion data. After searching the online platforms of scientific research studies, a total of 26 studies were found to be eligible for the estimation of the abortion risk difference. Despite the lack of studies with all the information required for analysis, a heterogeneity (Higgins  $I^2$ ) test below 20% was also used as an exclusion criterion in the meta-analysis. Eventually, 0% heterogeneity was derived for 12 studies (HIGGINS et al., 2003).

For each study, prior tabulation of data was required. Subsequently, a statistical package was used to calculate the mean difference, upper and lower limit of the abortion risk difference in seropositive animals compared to seronegatives. The analysis estimated the mean for abortion risk difference due to neosporosis in dairy cattle in Brazil (Table 1).

## RESULTS AND DISCUSSION

Currently, neosporosis is present in all Brazilian states and possibly causes major losses to dairy producers (LOCATELLI-DITTRICH et al., 2001; CORBELLINI et al., 2002; SARTOR et al., 2003; GALVÃO et al., 2011). One of the greatest challenges to the control of neosporosis in Brazil is the different forms of production. Extensive production results in greater contact of the animals with the contaminated environment. In addition, almost all properties have pet dogs with free access to all facilities while maintaining contact with the animals and pastures, and this favors horizontal transmission and insertion of the infectious agent in free herds (DUBEY et al., 2007).

The meta-analysis (Table 1) revealed that the mean abortion risk difference was 10.04% (0.091-0.118) for the dairy herds of Brazil, with the Higgins  $I^2$  value for heterogeneity of 0% ( $P=0.651$ ) fulfilling the assumption of homogeneity in the results (HIGGINS

et al., 2003). Calculation of the abortion risk difference is mostly unperformed in scientific papers addressing *Neospora caninum*. These frequently choose to calculate the odds ratio, which causes estimation errors when inserted into the economic model because they vary with a higher prevalence of the disease under study, consequently leading to erroneous estimates of the total cost (ZHANG et al., 1998).

Table 1 shows that there is no major difference in results among studies in relation to different diagnostic methods, this difference could be associated with variances in sensitivities and specificities of each method. The results of indirect immunofluorescence (IFA) ranged from 8.7% to 21.5% in different states, while those of ELISA resulted in a mean risk difference of 12% to 16%. Only the study by CORBELLINI et al. (2006) in Rio Grande do Sul used an immunohistochemistry method to obtain a 16% abortion risk difference, similar to the results of ELISA and IFA in the same state. From this result, the risk difference obtained cannot be explained only by the diagnostic method used, but by differences in the study area, animals, and time period.

In the actual setting of the milk production chain in Brazil, an average prevalence of 20% for neosporosis in dairy cattle and the presence of 22,803,519 milked cows in 2012 (IBGE, 2012) represents a total of 4,560,703 possible seropositive animals. Taking into account a 10.40% for neosporosis-specific abortion risk difference, there are 474,000 potential cases of abortion associated with *Neospora caninum*.

Data published by ANDERSON et al. (2000) and DUBEY et al. (2007) showed that *Neospora caninum* is less likely to cause abortion when the infection becomes endemic to the herd. According to these authors, what it's usually observed are outbreaks of abortions due to primary infection in herds without previous contact with the parasite, with a decrease of the episodes during subsequent lactation, possibly because of acquired resistance. These episodes may intensify in animals with a low immune status during pregnancy.

The abortion risk difference calculated in this study should be used in scenarios of endemic disease, considering that the scientific studies that led to this meta-analysis did not report outbreak situations characterized by an increase in the risk of abortion. For a specific abortion outbreak scenario, the risk difference and the number of cases will be higher than calculated, resulting in higher costs to the producer.

Unfamiliarity with the magnitude of the physical effects of neosporosis makes the measures of control and prevention are often not adopted.

Therefore, the transmission of neosporosis occurs on a large scale with minimum control, leading to chronic and widespread disease in national dairy herds and causing significant economic losses.

Therefore, one of the challenges encountered during mathematical modeling of the cost associated with a disease is an estimation of these physical effects that is compatible with local realities. Knowledge of the magnitude of the impact of neosporosis on the dairy business, particularly by calculating its main effect on the risk of abortion in Brazil, is essential to implement control and cost-effective preventive measures at the study site (BENNETT et al., 1999; DUBEY et al., 2007).

The economic model for the total cost associated with neosporosis should cover the following parameters (HADDAD et al., 2005; DUBEY et al., 2007): cost of abortion, which is important to calculate the abortion risk difference, along with indirect costs such as those for veterinary aid, laboratory diagnosis, and calf loss; premature culling of animals because of episodes of abortions, low production, and various reproductive problems, with constant efforts to determine a cost-effect relationship; cost of animal replacement after abortion and involuntary culling because of neosporosis; decrease in milk production, either directly due to the disease or indirectly due to increased calving intervals caused by abortions and reproductive problems.

The economic modelling of abortion should cover and quantify the number of animals at risk, prevalence of *Neospora caninum* in locations to be studied, indirect costs of abortion, treatment and veterinary care, market value of the aborted calf, and the most difficult and sensitive variable to be estimated, which is the increased abortion risk in seropositive animals. (HADDAD et al., 2005; DUBEY et al., 2007; REICHEL et al., 2013).

REICHEL et al. (2013) estimated losses due to abortion caused by neosporosis in dairy herds in Brazil at 51 million USD, with a specific risk of abortion of 14.30% (0.6%-39.40%). This value was obtained from the odds ratio estimates for the other countries involved in the study, which may have led to an error in the estimate mainly because of different local circumstances. HADDAD et al. (2005) estimated the abortion risk at 18% (SD, 0.01) through a meta-analysis conducted in Canada, which was a higher value than that resulting in this study (10.04%). This difference can be explained by differences in genetics, systems of management, production and values of the Canadian animals.

This scenario represents major losses when it is considered that the base of the milk

production chain in Brazil is sustained, in milk volume and number of establishments, by the family farms, with low productivity and technology, low economic power, and insufficient access to rural credit, creating a very high dependence on the reproductive and productive success of their herds (IPEA, 2014). Moreover, this estimate is based on the cost incurred by only one specific disease, which leads to the affirmation that the losses to the milk producer, taking into account multifactorial diseases within a herd, are significantly high.

Although the economic impact caused by episodes of abortion is the largest in terms of the neosporosis related cost to dairy farmers, the studies by HADDAD et al. (2005) and DUBEY et al. (2007) reported that, to estimate the total direct cost associated with the neosporosis, it must be considered not only the loss caused by abortion but also factors such as: the decline in milk production, premature culling of animals, transmission rates, and various reproductive disorders, which should be analyzed using Brazilian data.

## CONCLUSION

The estimated abortion risk difference due to *Neospora caninum* infection in dairy cows in Brazil was 10.04% (0.091-0.118). This data is unpublished in Brazil and represents the prospect of more reliable calculations for the cost of the disease in dairy herds. The model for the cost of this disease will enable the use of cost-benefit analysis aimed to be the best control and preventive measures for the disease, resulting in an actual increase in the income of milk producers in Brazil.

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