The Use of Beef Bull Semen Reduced the Risk of Abortion in *Neospora*-seropositive Dairy Cows

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Summary
There is an evidence that the epidemiology of neosporosis differs in dairy and beef cattle, such that beef cattle carry a lower risk of abortion. The aim of the present study was to establish whether artificial insemination using semen from beef bulls could reduce the risk of abortion in dairy cows seropositive for the *Neospora caninum* parasite. Our study was based on yearly serological screening for neosporosis and on the confirmation of *Neospora* infection in aborted fetuses in two high-producing dairy herds with a mean 28% seroprevalence of *N. caninum* antibodies. The study population comprised of 273 pregnancies in seropositive animals: 156 pregnancies monitored after insemination using Holstein–Friesian semen and 117 after insemination using beef bull semen. Abortion rates for these animals were 28.2% (77 of 273), 34.6% (54 of 156) and 19.7% (23 of 117). Logistic regression analysis indicated no significant effects of lactation number and previous abortion on the abortion rate. Based on the odds ratio, a 1-unit increase in the *Neospora* antibody titre yielded a 1.01-fold increase in the abortion rate. The likelihood of abortion was two times higher for cows in one of the two herds and 2.8 times lower (one of 0.36) for pregnant cows inseminated with beef bull semen rather than Holstein–Friesian semen. Our results indicate that the use of beef bull semen can reduce the risk of abortion in dairy cows, and suggest that annual screening for neosporosis, specifically the antibody titre to the protozoan, could be an useful predictor of abortion risk in reproductive health programmes.

Introduction

*Neospora caninum* is an obligate intracellular protozoan, recognized in the last decade as a cause of bovine abortion worldwide (Dubey and Lindsay, 1996; Dubey, 1999; Anderson et al., 2000). Abortion caused by neosporosis is a significant cause of economic loss for the dairy industry and there is evidence that *Neospora* infection increases the calving interval and the culling rate on dairy farms (Thurmond and Hietala, 1996, 1997). The incidence of *N. caninum*-associated abortion peaks during the fifth to the seventh month of gestation (Anderson et al., 1991; Thornton et al., 1991; Wouda et al., 1997) and ranges from 3 months to term (Anderson et al., 1991; Barr et al., 1991; Thornton et al., 1991; Otter et al., 1995; Wouda et al., 1997; Hattel et al., 1998). A *Neospora*-seropositive cow is more likely to abort than a seronegative cow (Anderson et al., 1995; Moen et al., 1998; Davison et al., 1999; Hietala and Thurmond, 1999) to the extent that prospective serological tests in dairy herds have been used to predict abortion (Paré et al., 1997). In previous studies performed in north-east Spain, we found that seropositive cows in infested herds had a 12- to 19-fold higher risk of abortion than seronegative cows (López-Gatius et al., 2004a, b).

There is some evidence that the epidemiology of neosporosis varies in dairy and beef cattle. Several studies have shown a lower prevalence of infection in beef cattle compared with dairy herds. In Spain, the proportions of cows with antibodies to *N. caninum* recorded for several herds of beef and dairy animals were 17.9% (306 of 1712) and 35.9% (402 of 1121) respectively (Quintanilla-Gozalo et al., 1999). In Argentina, seroprevalence rates for cows without reproductive diseases of 4.7% (19 of 400) for beef cattle and 16.6% (174 of 1048) for dairy cattle have been reported. Moreover, of 966 serum samples from aborted cows, 18.9% (41 of 216) were positive for the protozoan in beef herds and 43.1% (323 of 750) were positive in dairy herds (Moore et al., 2002). These differences are mainly attributed to the management differences between dairy and beef cattle. Intensive herd management has been associated with increased seroprevalence for *N. caninum* (Sanderson et al., 2000; Ortranto et al., 2003). Besides, a lower risk of abortion has been noted in infected beef cows compared with dairy cows (De Meerschman et al., 2000). In addition, these authors found significantly more prominent and extensive intracerebral lesions in dairy cattle than in beef cattle fetuses (De Meerschman et al., 2002). The present study was designed to establish whether insemination with semen from beef bulls reduces the risk of abortion in *Neospora*-seropositive dairy cows.

Materials and Methods

Cattle and herd management

This study was performed over a 2-year period (1 January 2002 to 31 December 2003) on two commercial dairy herds in north-east Spain with previously confirmed diagnosis of *N. caninum* infection in aborted fetuses. The two herds comprised 620 mature Holstein–Friesian cows (180 and 440 animals respectively). Reared within the herds and kept in open stalls, the
cows calved all year round and were milked three times daily. For the study period and for both herds, mean annual milk production was 10,600 kg per cow and the culling rate was 28%. All animals were tuberculosis and brucellosis free, as shown by yearly tests from 1985 to 2004. Vaccination programmes were undertaken for the prevention of bovine virus diarrhoea and infectious bovine rhinotracheitis. Modified live vaccines were applied to animals between 6 and 8 months of age, whereas killed vaccines were administered to pregnant animals during month 7 of gestation, for every gestation period. Further, parous cows which did not become pregnant on day 150 post-partum received a killed vaccine.

The herds were subjected to a reproductive health programme involving weekly checks. The reproductive tract of each animal was examined by palpation per rectum within 34–40 days postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time were treated until resolved or until culling. All the animals were bred by artificial insemination (AI). Seronegative animals received Holstein–Friesian semen at AI, whilst, in the chronological order of oestrus detection, one in three inseminations applied to seropositive cows was conducted using beef bull semen from Limousin or Belgian blue bulls (insemination policy of the herds allowed the use of beef semen in only one of the three AI, not every other AI). Holstein–Friesian semen was used in the remaining AI applied to seropositive cows. The number of dairy bulls, Limousin and Belgian blue bulls used were 22, two and three respectively.

Pregnancy diagnoses were performed on day 34 post-insemination by transrectal ultrasound, and by palpation per rectum on days 90 and 180. As from day 90, animals were visually inspected daily for signs of abortion until parturition and only parous cows diagnosed pregnant on day 90, 0–365 days after serological analysis were included in the study. Data from seronegative animals were recorded and used as reference data. The final study population only included Neospora-seropositive cows. Heifers were not included.

Serological diagnosis

Serological analysis for Neospora infection was performed during annual screening for brucellosis. Blood samples were centrifuged and sera stored at −20°C until the time of analysis. Sera were tested for antibodies against N. caninum using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Civitest® anti-Neospora; Hypraa, Girona, Spain), based on the whole tachyzoite lysate of Neospora NC-1. This test, previously validated by the present authors (López-Gatius et al., 2004a), was performed according to the manufacturer’s instructions and a value of ≥6.0 taken to denote seropositivity. Briefly, the validation characteristics of the test kit included level of agreement between two serological tests: Civitest and Idexx (Herd check® anti-Neospora; Idexx Laboratories, Madrid, Spain). The two ELISA techniques were performed on samples collected from 3400 animals and showed a Kappa value of 0.94, indicating a very high level of agreement between the tests.

Histopathology of aborted fetuses

Fifteen aborted fetuses were submitted for laboratory analysis: 11 from seropositive cows and four from seronegative cows.

We looked for evidence of protozoal lesions in haematoxylin–eosin stained tissue. The presence of N. caninum was established by a specific immunohistochemical procedure (Lindsay and Dubey, 1989) and specific Ne5 PCR performed on brain tissue (Liddell et al., 1999, Almería et al., 2002).

Statistical analysis

Abortion data were obtained from cows that were diagnosed pregnant during the study period. Regression analyses were conducted according to the method of Hosmer and Lemeshow (1989) by the logistic procedure (SAS., 2001). Basically, this method involves five steps as follows: (i) preliminary screening of all variables for univariate associations; (ii) construction of a full model using all the variables found to be significant in the univariate analysis; (iii) stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; (iv) evaluation of interactions among variables and (v) assessment of model fit using Hosmer–Lemeshow statistics. Variables with univariate associations showing P < 0.25 were included in the initial model. We continued modelling until all the main effects or interaction terms were significant according to the Wald statistic at P < 0.05.

Logistic regression analyses were performed on data from each animal, using abortion as the dependent variable and herd, Neospora antibody titre (at the last screening prior to pregnancy diagnosis), semen providing breed (Holstein–Friesian versus beef), previous abortion (abortion registered before the current pregnancy) and lactation number as independent factors. Insemination with Holstein–Friesian semen and previous abortion were considered dichotomous variables, where 1 denotes presence and 0 denotes absence. The Neospora antibody titre and lactation number were coded as continuous variables. The herd was considered as a class variable. All values are expressed as the mean ± SD.

Results

The seroprevalence of N. caninum antibodies for both herds was 28%. In total, 168 animals were recorded as seropositive, the mean titre being 54.3 ± 33.2 units (range 6.1–140.4). Of the 741 seronegative animals that became pregnant, 25 (3.4%) aborted, whereas abortion occurred in 77 (28.2%) of the 273 pregnancies recorded in seropositive animals.

All 11 fetuses from seropositive animals submitted for laboratory analysis showed lesions indicative of Neospora infection, whilst no lesions related to neosporosis were found in four fetuses from seronegative animals.

The final population study was comprised of 273 pregnancies in seropositive animals: 156 pregnancies recorded after AI using Holstein–Friesian semen and 117 after AI using beef bull semen (48 inseminations using semen from Limousin bulls and 69 from Belgian blue bulls). In four dams, a mummified fetus was detected during pregnancy diagnosis on day 180. These cows were recorded as having aborted on day 180 of gestation. The mean period in which abortion was registered was within 160 ± 33.9 days of pregnancy, ranging from 101 to 259 days.

Logistic regression analysis indicated no significant effects of lactation number and previous abortion on the abortion rate. The mean lactation number was 2.4 ± 1.6 lactations, ranging from one to eight lactations. Abortion rates for animals with
or without previous abortion were 33.3% (12 of 36) and 27.4% (65 of 237) respectively. Table 1 shows abortion rates according to the variables selected by the logistic procedure. Based on the odds ratio, a 1-unit increase in Neospora antibody titre yielded a 1.01-fold increase in the abortion rate. The likelihood of abortion was twice greater for cows in one of the two herds and 2.8 times (one of 0.36) lower for pregnant cows inseminated with beef bull semen compared with Holstein–Friesian bull semen. Mean antibody titres for aborting and non-aborting cows were 62 ± 31.5 and 51.3 ± 33.5 units respectively. Abortion rates for animals becoming pregnant in response to AI with beef semen were 19.7% (23 of 117): 16.7% (eight of 48) and 21.7% (15 of 69) for Limousin and Belgian blue bulls, respectively, whereas 34.6% (54 of 156) of the pregnant animals inseminated with Holstein–Friesian semen suffered abortion.

**Discussion**

To our knowledge, the incidence of abortion in Neospora-seropositive dairy cows following pregnancy achieved using beef bull semen has not been previously analysed. In our work conditions, the use of Holstein–Friesian semen proved to be a risk factor for pregnancy loss, the likelihood of abortion decreasing 0.36-fold with the use of beef bull semen.

Neospora caninum-infected cows show a high very rate of transplacental transmission (Paré et al., 1997; Wouda et al., 1998; Camargo et al., 2006; Piegari Fioretti et al., 2003). This rate has been estimated to be as high as 95% (Devos et al., 1999). Effectively, in chronically infected dairy cattle, most aborted fetuses show signs of infection. De Meerschman et al. (2000) hypothesized that transplacental transfer of the parasite during pregnancy can be reduced in beef cattle, compared with dairy cattle. Additional studies on cross-breeds aborted fetuses or newborn calves from infected cows would clarify this point.

On the contrary, placental function might be favoured in cross-breed pregnancies. Pregnancy-associated glycoproteins (PAG) are a multigenic family belonging to the aspartic proteinase superfamily (Xie et al., 1991). These proteins are abundantly expressed in the outer-cell layer of the placenta of artiodactylids such as porcine and ruminants (Szafranska et al., 1995; Xie et al., 1997; Garbayo et al., 2000). Although their function is still unknown, PAG levels in maternal blood have been used for pregnancy diagnosis and as a marker for placental/fetal well-being (Skinner et al., 1996; Zarrouk et al., 1999a,b). Indeed, a protective mechanism against rejection has been suggested based on the finding that peripartum PAG levels in cows carrying fetuses of a different breed were higher than levels in cows bearing fetuses of their own breed (Zoli et al., 1992). Similarly, domestic goats carrying Spanish ibex fetuses were found to have PAG concentrations that were 10 times higher than intraspecies gestations of either domestic goats or Spanish ibex (Fernández-Arias et al., 1999). Thus, the greater genetic distance between the fetus and mother was associated with higher PAG concentrations. Whether the present findings in dairy cows bred for beef bull semen reflect the same situation will be the subject of our next study.

As a reference figure, we recorded a 3.4% abortion rate in seronegative herds. This incidence is lower than the 5% abortion rate described for dairy cattle from 90 to 265 days of gestation (Forar et al., 1996). Thus, the 28.2% abortion rate observed here for the seropositive animals suggests a strong association between N. caninum infection seropositivity and abortion, in agreement with previous reports (Dubey et al., 1997; Paré et al., 1997; Moen et al., 1998; Mainar-Jaime et al., 1999; Stenlund et al., 1999; Moore et al., 2003) and with results in our geographical area (López-Gatius et al., 2004a,b). Our confirmation of infection in 11 aborted fetuses reinforces the causal link between N. caninum infection and abortion in the present study. Further, a close association between antibody titres and abortion was observed: each unit increase in antibody titre resulted in a 1.01 increase in abortion rate. We blood-sampled both the entire herd at the time of yearly brucellosis screening. Although it is possible that the time between N. caninum screening and AI could have had an influence on the main variable, our results support the proposal by Quintanilla-Gorozl et al. (2000), that the antibody titre (and not only seropositivity) could be used as a cost-effective predictive tool to identify animals carrying a high risk of abortion in herds with a high seroprevalence for Neospora caninum.

Previous abortion was not a risk factor for abortion. Perhaps the small size of the study population masked any possible effect of this factor; only 36 animals were registered pregnant following a previous abortion. The effect of the herd (the risk of abortion was two times greater in one of the two herds) could be multicausal. Our study population was made up of high milk yielding cows and the intense metabolic stress suffered by these high production animals, among other stressful causes, can compromise their immune response.

In conclusion, our results indicate that the use of beef bull semen can significantly reduce the risk of abortion in dairy cows and suggest that annual screening for neosporosis, specifically the antibody titre, could be an useful predictor of abortion risk.

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<th>95% Confidence interval</th>
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Likelihood-ratio test = 306.4; 3 d.f., P < 0.0001.
Hosmer and Lemeshow goodness-of-fit test = 5.17; 8 d.f., P = 0.74 (the model fits).
References


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