Neosporosis in naturally infected pregnant dairy cattle

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A B S T R A C T

Neosporosis caused by caused by the apicomplexan parasite Neospora caninum is one of the major causes of infectious abortion in bovines worldwide. A long-term prospective study was performed in a dairy herd endemic for N. caninum in order to analyze the impact of neosporosis on the proportion of aborting cows. A total of 1078 pregnant cows were tested for presence of antibodies and the proportion of abortions was calculated. The overall seroprevalence of N. caninum found in the herd was 35.5%. The percentage of abortions in seropositive cows was 3 times higher than in their seronegative counterparts (21.6 and 7.3%, respectively). No statistically significant association was found between the antibody level of positive during pregnancy and the proportion of aborting cows. However, 41.2% of the dams with antibody titers of 1:12,800 aborted. The risk of abortion for such dams was 2.7 times higher than for other seropositive cows which had lower titers of antibodies (p = 0.0072). In the follow-up examinations of the seropositive cows during several pregnancies, the overall percent of abortions observed was significantly higher than in seronegative individuals (49.3 and 16.9%, respectively: p < 0.0001). Moreover, the proportion of repetitive abortion observed was 5 to 1 (17.4 and 3.5%) in seropositive and seronegative dams, respectively (p < 0.001). The rate of vertical transmission in positive dams was 61.0% and it appeared to be directly associated with antibody levels: the higher the titer in the dams during pregnancy, the higher the percentage of sero-positivity in their calves. Increased proportion of abortions was observed in seropositive cows both in summer and winter in comparison with spring and autumn. It was found that in seropositive cows, an increased number of pregnancies, which was directly related to the age of the dam, has been associated with an increased number of abortions.

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1. Introduction

The protozoan parasite Neospora caninum is generally accepted to be major cause of infectious abortion in cattle worldwide (Dubey and Scharas, 2011; Reichel et al., 2013). Cattle become infected horizontally by consumption of infective oocysts (McAllister et al., 1998) or vertically by in utero transmission of tachyzoites during pregnancy (Anderson et al., 2000). Congenital infection occurs in consecutive pregnancies and has been shown to be important
in the spread of the disease. It is generally accepted that vertical transmission is the major mode of transmission in cattle (Paré et al., 1996; Schares et al., 1998).

In pregnant cows, infection with *N. caninum* can lead to several different scenarios: early fetal death and re-absorption; abortion, stillbirth or parturition of a deformed calf; and birth of a clinically normal but infected offspring (Dubey, 1999). The apparently healthy, congenitally infected calves remain persistently infected for life and might undergo abortions in subsequent pregnancies (Thurmond and Hietala, 1997; Fioretti et al., 2003; Pabón et al., 2007). Infected cows do not exhibit clinical signs except for abortion; therefore, the early diagnosis and application of preventive measures to control neosporosis are limited.

The pathogenesis of neosporosis in cattle is not fully understood (Dubey et al., 2006). Factors that are likely to affect the severity of the disease and the outcome of pregnancy include: the timing of the infection; the duration of parasitemia in the pregnant cow; the dynamics of the maternal immune response in pregnant dams; and the immune capacity of the fetus (Innes et al., 2005; Innes, 2007; Rosbottom et al., 2011). However, it is known that higher risk of abortion and recurrent abortions are associated with seropositive cows (Dubey et al., 2007). Previous studies showed that the risk of abortion is three to 23 times higher in *Neospora*-seropositive cows than in seronegative cows (Nogareda et al., 2007). Moreover, in persistently infected pregnant cows, endogenous transplacental infection was associated with a significant increase in maternal antibodies (Guy et al., 2001). However, fluctuations in antibody level during pregnancy have been observed in both aborting and non-aborting chronically infected cows (Nogareda et al., 2007) and the reason causing some seropositive cows to abort and others not is still obscure. In the present paper, we describe a long-term prospective study performed in an intensive dairy herd endemic for *N. caninum*, which emphasizes the impact of neosporosis on abortions of naturally infected cows.

2. Materials and methods

2.1. Cattle and herd management

The study was performed from January 2007 to December 2011 in a Holstein–Friesian dairy herd located in central Israel, with 500 dams and a history of 15% *Neospora*-associated abortions. The herd is kept under an intensive maintenance system. The access of other animals to the herd, including canids, is strictly minimized. The way in which neosporosis was introduced into the herd is unknown. There is no seasonal breeding management in the herd; artificial insemination is performed around the year. All the animals were bred by artificial insemination. On day 42 post insemination, pregnancy examinations were performed routinely by palpation per rectum (fetal-membrane slip method). Pregnancies were reconfirmed on day 110–130 post insemination, before serum sampling. All animals were vaccinated against brucellosis, food and mouth disease (FMD) bovine respiratory syncytial virus (BRSV), rinderpest virus (RPV), infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) and clostridiosis.

Early culling management based on the *Neospora* serostatus, reproductive failure or number of abortions was not practiced in this herd. According to the clinical veterinarian, the only criterion for culling was economic consideration based on the level of milk production or aging of cows, irrespective of abortion events.

2.2. Indirect fluorescent antibody test (IFAT)

Blood samples were collected from coccygeal vein. After centrifugation at 1000 × g for 20 min, the serum was separated and stored at −20 °C, pending examination for the presence of antibodies to *N. caninum* by IFAT according to Shkap et al. (2002). An antibody titer of 1:200 was considered as a borderline for this study; titers of 1:400 or higher as positive.

2.3. Experimental design

During five consecutive years (2006–2011), blood samples from pregnant cows were collected on the 110–130th day of gestation. Overall, 1078 pregnant dams were sampled; some of them during more than one pregnancy. The outcomes of the pregnancy, season of abortions, antibody titers during pregnancy and at parturition were recorded. The vertical transmission rate was estimated from the proportion of seropositive calves born to dams with known sero-status during pregnancy. The pre-colostral blood sampling of the calves on time was not technically feasible. Bleeding was performed only at 12–18 months in order to exclude detection of colostral antibodies in the test samples.

2.4. Statistical analysis

Statistical analyses were carried out with JMP software (JMP®, Version Macintosh. SAS Institute Inc., Cary, NC, 2000). The analyzed parameters related to pregnancy outcome: abortion or calving, with reference to the IFAT titers. The association of the *N. caninum* sero-status with the pregnancy outcome was determined by contingency Chi–square (χ²) test. The association of the pregnancy number and its outcome was determined by logistic-fit Chi square (χ²) test. The relative risk of abortion (RR) in seropositive dams was calculated as the percent of abortion in seropositive cows/percent of abortion in seronegative cows.

3. Results

3.1. Serological status of the herd

Examination of 1078 pregnant cows in a neosporosis-endemic herd revealed that 382 sera (35.5%) were positive. A borderline titer of 1:200 was found in 95 sera (8.8%), 601 sera (55.7%) had no antibody reactivity when diluted 1:200 and, therefore, were considered negative (Table 1). Of the 382 seropositive samples, 22% exhibited titers of 1:6400 or higher.
Table 1

N. caninum antibody titer distribution at 110–130 days of gestation (n = 1078).

<table>
<thead>
<tr>
<th>Titer at 110–130 days of pregnancy</th>
<th>Number of dams (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>601 (55.7)</td>
</tr>
<tr>
<td>1:200</td>
<td>95 (8.8)</td>
</tr>
<tr>
<td>1:400</td>
<td>57 (5.3)</td>
</tr>
<tr>
<td>1:800</td>
<td>115 (10.7)</td>
</tr>
<tr>
<td>1:1600</td>
<td>45 (4.2)</td>
</tr>
<tr>
<td>1:3200</td>
<td>81 (7.5)</td>
</tr>
<tr>
<td>1:6400</td>
<td>31 (2.9)</td>
</tr>
<tr>
<td>1:12,800</td>
<td>53 (4.9)</td>
</tr>
</tbody>
</table>

3.2. Association between N. caninum seropositive titer and proportion of aborting cows

The proportion of aborting cows in seropositive pregnant cows was 21.6%, which is significantly higher (p < 0.0001) than in seronegative ones (7.3%). The relative risk of abortion (RR) for the seropositive dams was 2.9.

There was no statistically significant association between the antibody titer and the proportion of abortions observed among seropositive cows (Fig. 1A). However, in dams with titer of 1:12,800 (Fig. 1B), the proportion of aborting cows was significantly higher (41.2%) compared to that observed in all other positive cows tested (15.4%), (p = 0.0072).

Table 2 presents antibody titers tested on the 110–130 day of gestation and at the end of the gestation in 529 dams. At the first examination, 26.8% of the dams were seropositive, 8.7% showed a borderline reactivity and 64.5% were seronegative. At the end of the pregnancy, out of 142 positive dams, 126 (88.8%) dams remained positive, 11 were found negative (7.7%) and 5 (3.5%) had borderline titer. Among the 11 cows that appeared seronegative at the end of pregnancy, the antibody titer ranged from 1:400 to 1:1600. One of these dams, which had a titer of 1:1600, aborted. Among the group of dams seronegative at the 110–130 gestation day (n = 341), 325 remained negative, 10 (3.0%) seroconverted to a positive result, and 6 (1.7%) depicted a borderline titer. Among the animals with the borderline titer at the first testing (n = 46), 16 exhibited increased seropositivity (titers of 1:400 to 1:6400 were found), 12 (26.0%) remained with borderline reactivity, while 18 (39.1%) became negative. Among the dams which were seronegative or with borderline antibody reactivity that became seropositive during the second or third term of gestation, no abortions were observed.

Table 2

Comparison of the sero-status of cows during and at the end of pregnancy (n = 529).

<table>
<thead>
<tr>
<th>IFA titer at the end of pregnancy (%)</th>
<th>IFA titer at 110–130 days of pregnancy (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Borderline</td>
</tr>
<tr>
<td>325 (95.3)</td>
<td>6 (1.7)</td>
</tr>
<tr>
<td>18 (39.1)</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>11 (7.7)</td>
<td>5 (3.5)</td>
</tr>
<tr>
<td>341 (65.4)</td>
<td>10 (3.0)</td>
</tr>
<tr>
<td>46 (8.7)</td>
<td>16 (34.8)</td>
</tr>
<tr>
<td>142 (27.9)</td>
<td>81 (15.5)</td>
</tr>
<tr>
<td>142 (27.9)</td>
<td>31 (6.4)</td>
</tr>
<tr>
<td>142 (27.9)</td>
<td>53 (10.4)</td>
</tr>
</tbody>
</table>

3.3. Proportion of aborting cows in consecutive pregnancies

A total of 255 cows were observed for up to five pregnancies; 69 of them were seropositive, 171 seronegative and 15 changed their sero-status during the experiment. Among seroconverted animals, six (2.4%) cows that were found negative in the first examination became positive during the experiment; four were initially found positive, but became negative at two or three subsequent time points; five cows showed fluctuating titers, being mostly positive, but negative at some points. The 15 seroconverted animals were excluded from the analyses in consecutive pregnancies. As shown in Table 3, in 69 seropositive dams, a total of 34 (49.3%) aborted. In contrast, abortion occurred in 29 out of 171 (16.9%) seronegative dams (p < 0.0001). In seropositive cows, the number of abortions became higher as the number of observed pregnancies increased from 41.1% abortions at 2 gestations to 45.8% and 77.7% at 3 and 4 pregnancies, respectively. Among the 171 seronegative cows, the percentage of abortion was 15.1%, 13.5% and 36.4% when followed for 2, 3 and 4 pregnancies, respectively. Only four cows (2 positive and 2 negative) were followed up during five consecutive years. All of these cows had aborted.

Twelve out of 69 (17.4%) seropositive cows experienced recurring abortions, while in seronegative cows such abortions were observed only in six out of 171 animals (3.5%). Thus, the risk of recurrent abortions in seropositive dams was 5 times higher (p = 0.0006) than that of seronegative ones (Table 3).

3.4. Vertical transmission rate

Serological tests of 199 paired calves and dams are summarized in Table 4. Based on the antibody titers, the overall rate of vertical transmission in positive dams was estimated to be 61%. Vertical transmission ranged from 28.6% in borderline positive dams up to 100% in highly positive cows with titers equal to or above 1:6400. In seronegative dams, 11.2% of the offsprings were found seropositive at the first pregnancy. In dams with borderline titer, the vertical transmission percentage was higher than that in seronegative ones, but the difference value was only marginally significant (p = 0.0570). The overall percentage of seropositivity in the calves was increasing in correlation with the titer of the dams during pregnancy.

3.5. Effect of pregnancy number on proportion of aborting dams

The number of abortions vs the number of pregnancies was recorded in the follow up of a total of 356 pregnancies. Logistic fit results show that among seropositive dams (n = 234), the proportion of aborting dams augmented significantly (p = 0.0053) in parallel to the increasing number of the pregnancy, whereas among seronegative dams (n = 122), the number of the pregnancy did not affect the proportion of aborting dams (Fig. 2).
3.6. Influence of season on proportion of aborting dams

The season of the abortion or calving was recorded for 888 pregnant cows. Month-to-month variations in the percentage of aborting dams were observed in both seropositive and seronegative dams. The number of abortions over the months varied from 0% (in April) to 37.5% (in June) in seropositive dams, and from 1.3% (in February) to 13.9% (in December) in seronegative dams, but the differences were not statistically significant. However, the

### Table 3

Percent of abortions in cows observed during several pregnancies.

<table>
<thead>
<tr>
<th>Number of pregnancies observed</th>
<th>Total number of dams</th>
<th>Seropositive</th>
<th>Seronegative</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dams</td>
<td>Aborting dams (%)</td>
<td>Dams</td>
</tr>
<tr>
<td>2</td>
<td>133</td>
<td>34</td>
<td>14 (41.1)</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>83</td>
<td>24</td>
<td>11 (45.8)</td>
<td>59</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>9</td>
<td>7 (37.7)</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>2</td>
<td>2 (100)</td>
<td>2</td>
</tr>
<tr>
<td>Overall abortion</td>
<td>240</td>
<td>69</td>
<td>34 (49.3)</td>
<td>171</td>
</tr>
<tr>
<td>Recurrent abortion</td>
<td>240</td>
<td>69</td>
<td>12 (17.4)</td>
<td>171</td>
</tr>
</tbody>
</table>

### Table 4

Vertical transmission of *Neospora caninum* based on antibody titer analyzed by IFAT.

<table>
<thead>
<tr>
<th>Sero-status of the dams (at pregnancy)</th>
<th>Sero-status of the calf (at 12–18 months)</th>
<th>p Value^a^</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n = 125)</td>
<td>107 (85.6)</td>
<td>4 (3.2)</td>
</tr>
<tr>
<td>Borderline (n = 14)</td>
<td>9 (64.3)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>1:400–1:800 (n = 35)</td>
<td>14 (40)</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td>1:1600–1:3200 (n = 17)</td>
<td>4 (23.5)</td>
<td>0</td>
</tr>
<tr>
<td>≥1:6400 (n = 7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total seropositive (n = 59)</td>
<td>18 (30.5)</td>
<td>5 (8.5)</td>
</tr>
</tbody>
</table>

^a^ IFA borderline is refer to dams with antibody titer of 1:200.

^b^ p Value was calculated comparing to negative dams.
percent of abortions in seropositive dams during summer and winter (30.7% and 26.8%, respectively) was notably and significantly higher ($p = 0.0140$) than during the fall and the spring (15.5% and 16.7%, respectively). The only statistically significant difference in seronegative dams ($p = 0.01$) was found between the summer and the fall (10.9 and 3.9%, respectively).

4. Discussion

Seroprevalence of *N. caninum* in dairy herds worldwide ranges from 3.0 to 65.0% (Dubey et al., 2007). Aborting cows in Israeli dairy herds depicted 51.4% seropositivity for *N. caninum*. 18% of the abortions were associated with neosporosis and confirmed by serological and molecular assays in the aborted fetuses (Mazuz et al., 2011). In the present study, which was performed in a closed intensively bred dairy herd endemic for neosporosis, the seroprevalence among pregnant dams was 35.5%. The proportion of aborting cows observed in seropositive dams was 3 times higher than that in seronegative ones (21.6 and 7.3%, respectively). These results are consistent with the reports from various countries showing abortion rates 2–19 times higher for seropositive compared to seronegative cattle (Paré et al., 1997; Davison et al., 1999; López-Gatius et al., 2004; Hall et al., 2005; Dubey et al., 2007). Reichel et al. (2013) describe higher abortion risk in *N. caninum*-seropositive dairy cattle. The risk ranged from 1.3 to 40.0 in various studies, with a median value of 3.5.

The IFAT during pregnancy was not directly associated with the risk of abortion. However, a significant increase in the proportion of aborting cows (41.2%) was observed in dams whose titer during pregnancy was 1:12,800. Our findings are compatible with those of Stenlund et al. (1999), who observed an association between high level of antibodies (1:12,800 in ISCOM ELISA) and increased frequency of abortions.

In the present study, a follow-up of 240 cows over 5 consecutive years showed that the total percentage of abortions in seropositive dams was 49.3%, compared to 16.9% in negative cows. Those numbers are indicating a 2.9-fold higher risk of abortion in seropositive dams than the risk in seronegative dams. Recurring abortions were observed in 17.4% of the seropositive dams, the risk of recurring abortion among seropositive cows was 5-fold higher than among seronegative ones. These findings are in accord with those of a long-term study by Pabón et al. (2007), which showed that *N. caninum* seropositivity was stable over time and that it was associated with high rate of repeated abortions.

The majority of dams tested during pregnancy and after parturition retained their initial seropositive (88.8%) or seronegative (95.3%) status, although antibody titer fluctuations were observed in seropositive dams. Similar fluctuations were reported by other sources (Conrad et al., 1993; Paré et al., 1997; Stenlund et al., 1999). We found that among cows with low antibody titer (1:200) during pregnancy, 39.1% became seronegative, and 34.8% became seropositive at the end of their pregnancy; meaning that in endemic herds this titer represents an unstable, varying and non-conclusive sero-status. In view of this finding, we suggest that cows with borderline titers require retesting, before management decisions associated with the sero-status of the dam are made.

With regard to vertical transmission, it appears that the percentage of infection in the offspring was directly correlated with the antibody level during pregnancy; all calves born to dams with titers of 1:6400 and above were found positive for *N. caninum* when tested at about 1 year of age. These results are consistent with previous reports, in which cows with high level or marked increase of antibodies during pregnancy were more likely to give birth to seropositive calves (Paré et al., 1997; More et al., 2009). Goodswen et al. (2013) concluded that despite the difference in the rate of vertical transmission observed in different studies, it was still evident that transplacental transmission was the major mode of infection in cattle. In our study, out of calves born to dams with borderline titer of 1:200, 64.3% tested either negative or borderline, and 28.6% were found seropositive. Similarly to Dubey et al. (2007) and Weston...
et al. (2012), we found that about 10% of the calves born to seronegative dams were found seropositive. The possibility of seropositive calves being born to seronegative dams has been discussed by; this phenomenon may be associated with fluctuations of antibody levels to below the detection limit during pregnancy, but it also could be a result of horizontal transmission. It is possible that at certain stages of the pregnancy the parasites are hidden and not exposed to immune surveillance and consequently no antibody response is elicited (Benavides et al., 2012). As mentioned above, the calves were tested at the age of 12–18 months and the possibility of horizontal transmission could not be entirely excluded, but it is unlikely due to the conditions in which the herd was kept; a closed farm with tight restrictions for canid presence. As seroconversion was found in only 2.3% of the herd over the 5 years of the study, we suggest that the horizontal transmission was negligible.


In the studied herd, *N. caninum*-seropositive dams appeared to have higher level of abortions in the summer and winter, although a slightly increased number of abortions were observed in seronegative cows during summer. According to Dubey and Schaeres (2011), seasonality and humidity might influence the risk of neosporosis abortion in chronically congenitally infected cows. It was also reported, that increase in rainfall and in cumulative number of days with mean relative humidity lower than 60% during the second trimester of gestation, increased the abortion rate in *N. caninum*-infected dams (López-Gatius et al., 2005; Yániz et al., 2010; Almería and López-Gatius, 2013). The central area of Israel, where the presently addressed herd is located, has a typical Mediterranean climate characterized by long, extremely hot, dry summers and relatively short, cool, rainy winters. In light of our present results, it appears that the seasons with stronger weather conditions impact — summer and winter — may act as stress factors eliciting changes in the immunological status of the dams and, thereby, facilitate the recrudescence of infection and abortion. Wouda et al. (1999), observed abortion epidemics during warm and humid summer in Netherlands herds. Management herd practice, such as temperature control by means of cooling/warming systems, might moderate the stress imposed on the animals in the dry summers and rainy winters and decrease the *Neospora*- associated abortion.

The influence of the age of the cows in *Neospora*- associated abortions was widely discussed, but there are no generally accepted conclusions. A greater risk of abortion was observed in heifers than in subsequent pregnancies (Thurmond and Hietala, 1997), but this contrasts findings, in which there was either no or a protective effect found in heifers compared with parous cows (Jensen et al., 1999; Hernandez et al., 2002). We observed that the proportion of aborting cows increased with increasing number of pregnancies. These results are consistent with some of the previous studies (Jensen et al., 1999; Wouda et al., 1999; Kashiwazaki et al., 2004), but contrast other studies which showed an opposite effect (Thurmond and Hietala, 1997; Pabón et al., 2007; Yániz et al., 2010). The putative reduc- tion of abortion rate associated with increasing age might be a consequence of adopted control measures, including selective culling of aborting seropositive dams at an early age (Thurmond and Hietala, 1997). Dubey et al. (2007) sug- gested that the influence of age on abortions would differ between neosporosis endemic and epidemic herds, with an increase of abortion rate being directly associated with the age of the cow in an epidemic herd, and indirectly associated with it in an endemic herd.

Persistently infected cattle represent the major problem in the control of cattle neosporosis. Chronically infected dams experience recrudescence of infection in consecutive pregnancies, with the risk of abortion being associated with the age, climate and other stress factors. In infected herds, control programs are based on decreasing the vertical transmission. Different measures of control include “test and cull” strategies, vaccination, and alternative reproductive management (Dubey and Schaeres, 2011). According to Almería et al. (2009), insemination of *N. caninum*-seropositive cows with beef bull semen, especially by semen of Limousine breed bulls, has been proposed to reduce the risk of abortion. Although various management measures to diminish losses due to neosporosis–associated abortions are applied, significant control of chronic and congenital infections in highly endemic herds is still limited or economically not tolerable. Further studies targeting the development of effective measures to prevent either abortions or vertical parasite transmission in persistently infected animals are of high priority.

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References


