

Technical Bulletin 2

The Evaluation of a New Vaccine, NeoGuard™ *Neospora caninum* Vaccine, as an Aid in Reducing Abortions in Healthy, Pregnant Heifers Challenged with *Neospora caninum*

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Summary Points

- Thirty-eight pregnant heifers 18 to 24 months of age were used in the study.
- Treatments were as follows:
 - 1) Control – non-vaccinates and
 - 2) NeoGuard™, *Neospora caninum* vaccine, 5 mL given subcutaneously, two injections, 21 days apart.
- All heifers used in the study tested serum-negative for *Neospora caninum* antibodies at the beginning of the study.
- NeoGuard™ was given on days 56 and 77 of gestation.
- All heifers were challenged with a *Neospora caninum* challenge preparation on day 95 of gestation.
- NeoGuard™ vaccinated heifers had an increase ($P \leq 0.05$) in sample to positive (S/P) ratios at the time of challenge and 28 days post-challenge when compared to control heifers indicating a marked immunological response to the vaccination.
- NeoGuard™ vaccinated heifers had fewer ($P < 0.05$) abortions than control heifers.
- NeoGuard™ is efficacious and safe when used in healthy, pregnant heifers as an aid in reducing abortions in cattle exposed to a challenge of *Neospora caninum*.

I. Summary

Thirty-eight pregnant heifers 18 to 24 months old were used in this study to determine the efficacy of NeoGuard™, *Neospora caninum* vaccine. This vaccine is indicated for use in healthy, pregnant cattle as an aid in the reduction of abortions caused by *Neospora caninum*.

Pregnant heifers used in the study were given two 5 mL doses, subcutaneously, of NeoGuard™ vaccine on days 56 and 77 of gestation. The control and NeoGuard™ heifers received a *Neospora caninum* challenge preparation on day 95 of gestation. All heifers were pregnancy-tested by palpation and/or ultrasound at each vaccination and challenge dose administration.

ELISA measurement of *Neospora caninum*-specific antibodies in pregnant heifers demonstrated that the S/P ratio was greater ($P \leq 0.05$) at the day of challenge (1.4 vs 0.10), and 28 days post challenge (2.5 vs 1.28) in NeoGuard™ vaccinated heifers when compared to control heifers.

Control heifers had an increase ($P < 0.05$) in the number of abortions when compared to NeoGuard™ vaccinated heifers when challenged with *Neospora caninum* (4 vs 0). Pregnant heifers vaccinated with NeoGuard™ delivered a greater number of healthy, normal calves than the control group following a *Neospora caninum* challenge (18 vs 11). The study determined that NeoGuard™ is safe and efficacious as an aid in reducing abortions in healthy pregnant cattle exposed to a *Neospora caninum*.

II. Introduction

Neosporosis, caused by the coccidian parasite *Neospora caninum*⁽¹⁾, is not a new disease but has only recently been recognized as a major cause of abortion in California dairy cattle⁽²⁾ as well as throughout the world⁽³⁾. Though abortion is the only clinical symptom exhibited by infected cows, the disease generates considerable economic losses due to aborted fetuses, costs associated with rebreeding, decreased lactation time, loss of milk yield and replacement of culls. Cases have been reported worldwide with an estimated 40,000 *Neospora caninum* induced abortions occurring in California cattle annually⁽⁴⁾. Significant reduction in average daily gain, live body weight at slaughter and hot carcass weight of beef cattle were associated with detection of antibodies to *Neospora caninum*⁽⁵⁾. Until identification of the organism⁽¹⁾ in 1988, *Neospora caninum* infections had been attributed to *Toxoplasma gondii* infections due to biological and structural similarities between the causative organisms. Although dogs are the definitive host of this parasite^(6,7), it frequently infects sheep, goats, deer, horses, buffalo, and bison. However the most economically devastating infections are in the livestock industry and in particular among cattle, which are serving as intermediate host in the life cycle of the *Neospora caninum*. The intracellular parasite *Neospora caninum* can be propagated to cattle via horizontal and/or vertical methods. In horizontal infections, cattle ingest contaminated feed and/or water with *Neospora caninum* oocysts shed in feces of dogs. The horizontal pattern of transmission is believed to cause a cluster of abortions or abortion storms. During vertical propagation, *Neospora caninum* can be transmitted transplacentally from an infected cow to the fetus, which is a cause of sporadic abortions consistent with the definition of an endemic pattern of abortions.

Despite the importance of bovine neosporosis, studies of the immune responses following experimental vaccination have only recently been reported. In cattle, inactivated and adjuvanted experimental formulations of the *Neospora caninum* vaccine elicited antibody⁽⁸⁾ and cell mediated immune responses⁽⁹⁾, i.e. *Neospora*-specific proliferation of peripheral blood mononuclear cells and production of interferon- γ .

The objective of this study was to determine the efficacy of NeoGuard™, *Neospora caninum* vaccine, for use in healthy, pregnant cattle as an aid in the reduction of abortion caused by *Neospora caninum*. The vaccination regimen tested was two, 5 mL, subcutaneous injections of NeoGuard™ given 21 days apart on days 56 and 77 of gestation.

III. Materials and Methods

Thirty-eight healthy, pregnant heifers of commercial beef breeds 18 to 24 months of age without *Neospora caninum*-specific antibodies were included in the study (Table 1). Animals were housed in dry lots with feed and water provided *ad libitum*. Heifers were confirmed to be pregnant by palpation and/or ultrasonography in the first trimester and were randomly divided by computer generated random assignment into two treatments 1) control – non-vaccinated and 2) NeoGuard™ given 21 days apart at 56 and 77 days of gestation.

Table 1. Study Design

	NeoGuard™	Control
Number of pregnant heifers at the start	19	19
Vaccination route of administration	subcutaneous	NA
Gestation day of 1st vaccination	56	NA
Gestation day of 2nd vaccination	77	NA
Gestation day of <i>Neospora caninum</i> challenge	95	95

NeoGuard™ was administered subcutaneously in two, 5mL doses 21 days apart. The initial injection of NeoGuard™ was given at day 56 of gestation using aseptic technique on the left side of the neck. The second (booster) injection, was given at day 77 of gestation on the right side of the neck.

All heifers (n=38) were challenged at 95 days of gestation with a *Neospora caninum* preparation. Following the *Neospora caninum* challenge, animals were observed for adverse reactions by the attending veterinarian and monitored for abortion when fed by animal technicians. Following the *Neospora caninum* challenge, one heifer from each treatment group was removed from the study. The removal of

heifers was not related to the treatments. One heifer from the NeoGuard™ treatment died of bloat and one heifer from the control group was diagnosed with abomasum ulcers. During days 21 to 80 post-challenge, observations for abortion were made four times daily. All animals were evaluated for health and pregnancy status at each vaccination and challenge by ultrasonography or palpation by the attending veterinarian. Sera samples were collected from all heifers before randomization and on the 56th, 77th, 95th days of gestation. Last blood samples were collected at 123 day of gestation i.e. 28 days post-challenge. Sera samples were tested by a commercially available, *Neospora caninum* Antibody ELISA test (HerdCheck™), a USDA-licensed test kit from IDEXX Laboratories Inc.

The *Neospora caninum*-specific antibodies in sera samples are expressed as sample to positive (S/P) ratios. Serum samples with S/P ratios of less than 0.50 are classified as negative and ratios greater than or equal to 0.50 are classified as positive for *Neospora caninum* antibodies.

Pregnancy data was analyzed by the "StatMost" program utilizing Chi-square analysis to determine differences between treatments. Serological results were analyzed by Mann-Whitney, non-parametric test, for treatment differences.

IV. Results

Subcutaneous administration of NeoGuard™ vaccine to healthy, pregnant heifers did not produce unwanted injection site reactions in this study. Pregnancy results from this trial support the efficacy of NeoGuard™ as an aid in reducing abortions in healthy, pregnant cattle caused by *Neospora caninum* (Table 2). Among the control group of 18 heifers, 7 calves were lost. Two were stillborn, one died within 24 hours of birth and four (22 %) were considered as being aborted as the result of the *Neospora caninum* challenge. Heifers vaccinated with NeoGuard™ on days 56 and 77 of gestation had a significantly reduced ($P < 0.05$) rate of abortion with none of the vaccinates aborting.

Of the four heifers aborting within the control group, 3 were confirmed as abortions while one was determined to have had a resorbed fetus. Placenta samples from each of the three aborted fetuses were positive for *Neospora caninum*. Histological analysis of the infected fetuses also showed a tissue cyst in one calf and infected brain tissue in another. There were no histology samples submitted for evaluation from the resorbed fetus. Histological examination of brain tissue showed no evidence of *Neospora caninum* infection in either of the stillborn calves or the calf dying within 24 hours of birth.

Table 2. Pregnancy results.

	NeoGuard™	Control
Number of pregnant heifers (start)	19	19
Number of heifers (end)	18	18
Number of abortions	0 ^a	4 ^b
Abortion rate, %	0%	22%
Number of stillborns	0	2
Stillborn rate, %	0%	11%
Number calves dead <24 h after birth	0	1
Number of live healthy calves	18	11

a,b Means in a row with different superscripts are different ($P < 0.05$).

Serological data indicates that all animals responded to vaccination and challenge (Table 3). On the day of challenge, ELISA S/P ratios were greater ($P < 0.05$) in NeoGuard™ vaccinated heifers when compared to control heifers (1.40 vs 0.10). Additionally, 28 days post-challenge, NeoGuard™ vaccinated heifers had greater ($P \leq 0.05$) S/P ratios when compared to control heifers. NeoGuard™ elicited a significant antibody response in vaccinated heifers as measured by ELISA and when expressed as S/P ratios. Additionally, the vaccinated heifers mounted a significant anamnestic response post-challenge.

Table 3. Serological responses.

	NeoGuard™	Control
Number of pregnant heifers at challenge	19	19
ELISA S/P ratios at beginning	0.1383	0.0933
ELISA S/P ratios at time of challenge	1.3952 ^a	0.0972 ^b
ELISA S/P ratios 28 days post-challenge	2.4970 ^a	1.2841 ^b

a,b Means in a row with different superscripts are different ($P \leq 0.05$).

V. Discussion

Results from the study demonstrate the efficacy of the NeoGuard™, *Neospora caninum* vaccine in reducing abortions in pregnant cattle exposed to the *Neospora caninum* challenge preparation. NeoGuard™ vaccinated heifers had a lower ($P<0.05$) number of abortions than the control group (0 vs 4). This vaccination regimen protected the NeoGuard™ vaccinated heifers from a *Neospora caninum* challenge while unvaccinated heifers suffered an abortion rate of 22%. The abortion rate in the control, non-vaccinated heifers is consistent with rates observed under field conditions^(10,11,12,13).

While there were three additional calves lost within the control group, two being stillborn and one dying within 24 hours of birth, there was no direct indication that these events were linked to the *Neospora caninum* challenge.

These results demonstrate that NeoGuard™, *Neospora caninum* vaccine, is efficacious in reducing abortions caused by *Neospora caninum* in healthy, pregnant heifers. The recommended vaccination regimen is to administer NeoGuard™ during the first trimester of gestation, 5 mL subcutaneously, followed by a second subcutaneous 5 mL dose 3-4 weeks later. Revaccination with two doses is recommended for subsequent pregnancies.

VI. Conclusion

The results of this vaccination challenge study demonstrate that the NeoGuard™ vaccine is safe when two doses are injected subcutaneously during the first trimester of gestation 21 days apart. Administering NeoGuard™ on days 56 and 77 of gestation in healthy, pregnant heifers did not result in unwanted injection site reactions and did not adversely affect pregnancy rates or the percentage of normal calves delivered when heifers were exposed to a *Neospora caninum* challenge. Moreover, the study demonstrated that NeoGuard™ in fact reduced ($P<0.05$) the number of abortions among heifers exposed to a *Neospora caninum* challenge.

VII. References

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