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Humoral immune responses and safety of experimentalformulations of inactivated Neospora vaccines

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Abstract Antibody titers to Neospora antigens ranged from 40 to 160 before vaccination, from 80 to 5,1202 weeks after the first dose of vaccination, and 320 to 40,9602 weeks after the second (booster) vaccination. A peak antibody titer of 40,960 was also detected 28 days after the booster vaccination among animals vaccinated with Neospora vaccine formulated with Bay R1005 adjuvant. In heifers inoculated with experimental formulations of Neospora vaccines, transient development of injection site reactions resulted in 1 out of 15 animals. This injection site reaction was not detectable 14 days after the first observation and measurements were made. We have also demonstrated that vaccines derived from tissue-culture-grown Neospora tachyzoites are safe and would be expected to be efficacious.

Introduction

Neospora caninum is a protozoan parasite that infects companion animals and livestock and has been associated with abortion in dairy and beef cattle worldwide. Bovine abortion is an important disease problem in the cattle industry because of significant negative economic impact on revenues from decreased milk production and calf loss. Abortion and neonatal morbidity in cattle induced by Neospora infection are an important cause of bovine abortions in the United States and an emerging significant problem worldwide. Infections involving Neospora were reported as early as 1984 in dogs in Norway, and today Neospora caninum is a newly recognized pathogenic protozoan parasite of livestock and companion animals. This intracellular parasite is now considered to be a major cause of abortion in cattle (Anderson et al. 1997). Dubey and co-workers found similar parasites to Toxoplasma gondii in dogs in the United States. They distinguished them from Toxoplasma, and in 1988 named the parasite N. caninum (Dubey et al. 1988a). Subsequently, Dubey and co-workers (1988b) isolated the parasite from a dog, grew it in cell cultures and in mice, and then induced neosporosis in experimentally inoculated dogs.

The Neospora parasites in dogs and cattle are different strains of the same species. The life-cycle of N. caninum is believed to resemble that of Toxoplasma. The definitive host of Neospora has been recently described, and dogs are a definitive host, shedding Neospora oocysts (McAlister et al. 1998). Until the complete life-cycle of Neospora has been elucidated and all definitive hosts identified, it will be difficult to make specific recommendations for prevention of infection. Natural infections have been observed in dogs, cattle, goats, horses, sheep, deer, and water buffalo. Because the oocyst stage of N. caninum has been recently identified and they are not readily available, tachyzoites are currently used to induce experimental neosporosis. Tachyzoites are not the natural source of infection for cattle; however, they are the natural source of fetal infection. Inoculation of pregnant cattle with cultured Neospora tachyzoites reproduced fetal lesions similar to those in natural infections. Thus, the causative agent of bovine fetal/neonatal infections is also a protozoan parasite isolated and identified as N. caninum, based on the morphologic similarity to those of N. caninum isolated from dogs. The etiologic role of bovine Neospora as a cause of abortion and congenital disease was described (Conrad et al. 1993a) and was confirmed by experimental reproduction of fetal disease with Neospora Bovine Parasite Abortion Isolate (BPA-1) and re-isolation of the parasite (Barr et al. 1994).

Bovine abortion and stillbirth due to Neospora have been reported worldwide and cause significant economic loss to farmers. An estimate suggests bovine neosporosis
may cause U.S. $35 million in annual losses to the California dairy industry (Barr et al. 1996, 1997) and the diagnosis continues to increase in the United States cattle industry, as well as worldwide (Barr et al. 1998). Until recently, no reliable diagnostic test kits for Neospora have been commercially available. The lack of this simple and reproducible diagnostic tool has been a major limiting factor in recognizing the importance of this disease worldwide. On 8 January 1998, the USDA issued a license for the ELISA test kit to the IDEXX Laboratories, and since then we have had an N. caninum antibody test kits which can confirm Neospora infection among aborted cows. The IDEXX ELISA test was developed to enhance Neospora testing capabilities by utilizing standardized reagents with defined test parameters, thus avoiding subjective test interpretations. Testing can be automated and results can be available in less than 2 h.

Because of the prevalence and economic importance of neosporosis, a vaccine that is safe and efficacious should be developed and made readily available to help reduce economic losses associated with abortions caused by Neospora infections in cattle. Moreover, because of the lack of pharmaceutical and biological control measures for neosporosis, initial study was conducted at Bayer research facility to determine the immune responses and safety of experimental formulations of inactivated Neospora vaccines.

The current study was undertaken to evaluate immunological responses in heifers injected with three experimental formulations of inactivated Neospora tachyzoite antigens. The previous study showed that pregnant cows experimentally infected with Neospora tachyzoites transmitted infection to their offspring (Ho et al. 1997); however, when these cows were re-bred and re-challenged they did not transmit infection to calves.

More importantly, that study demonstrated that a Neospora-specific indirect fluorescence antibody (IFA) titer of pregnant cows around 320 or above on the challenge day prevented fetal infection and provided protection from challenge. Therefore, due to the emergency situation being observed in the cattle industry and the lack of pharmaceutical and biological control measures for neosporosis, we at Bayer attempted to investigate the safety and potential immune responses of inactivated Neospora antigens.

Materials and methods

Eighteen heifers, 7–9 months of age, were purchased and allotted to four groups.

Cattle procured for this study had Neospora titers ≤160, as determined by IFA test. All 18 heifers were randomly assigned to three experimental groups and one control group. The three experimental groups comprised five heifers each (n = 5) and the control group comprised three heifers (n = 3). Vaccine was prepared at one dosage level of Neospora tachyzoites derived from two different tissue cultures. Each dose of inactivated Neospora tachyzoites was formulated with two different adjuvants and the same stabilizers.

Heifers from experimental vaccine groups were injected subcutaneously with 5 ml of one of the Neospora vaccine preparations and re-vaccinated 4 weeks later. Heifers from group 1 were vaccinated with 12 × 10⁸ Neospora tachyzoites derived from tissue cultures supplemented with 5% horse serum and formulated into 5 ml with 10% Havlogen adjuvant. Heifers of group 2 were vaccinated with 12 × 10⁸ Neospora tachyzoites derived from tissue cultures supplemented with 10% horse serum and formulated into 5 ml with 10% Havlogen adjuvant. Heifers from group 3 were vaccinated with 12 × 10⁸ Neospora tachyzoites formulated into 5 ml with Bay R1005 adjuvant. Control animals from group 4 were kept in the same lot as vaccinated heifers from experimental groups. Blood samples were collected bi-weekly from all heifers, and serum samples were tested by the IFA test for seroconversion to Neospora-specific antibody (Conrad et al. 1993b). Also, injection site reactions were recorded at bi-weekly intervals for the duration of the experiment, with the last observation at 28 days after booster vaccination. The injection site reactions were recorded in centimeters.

Results and conclusions

Antibody titers to Neospora antigens of all serum samples taken from 18 heifers 3 weeks prior to vaccination and at the day of vaccination were ≤160. The effect of the vaccination was assessed by bi-weekly IFA tests of their sera samples for antibody titer against Neospora. Sera samples taken bi-weekly from these heifers showed in the IFA test that all vaccinated animals generated a detectable level of specific antibody for Neospora antigens, especially after the second dose of vaccine. Antibody titers to Neospora of the vaccinated heifers, measured by IFA, ranged from 40 to 160 before vaccination, from 80 to 5,120 2 weeks after the first vaccination, and from 320 to 40,960 2 weeks after the second (booster) vaccination. All 15 heifers vaccinated with Neospora vaccines had fourfold increases in their titer of antibodies to Neospora by day 14 after the booster vaccination. All heifers from