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Research paper

Testing the *Sarcocystis neurona* vaccine using an equine protozoal myeloencephalitis challenge model

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ABSTRACT

Equine protozoal myeloencephalitis (EPM) is an important equine neurologic disorder, and treatments for the disease are often unrewarding. Prevention of the disease is the most important aspect for EPM, and a killed vaccine was previously developed for just that purpose. Evaluation of the vaccine had been hampered by lack of post vaccination challenge. The purpose of this study was to determine if the vaccine could prevent development of clinical signs after challenge with *Sarcocystis neurona* sporocysts in an equine challenge model. Seventy horses that were negative for antibodies to *S. neurona* and were neurologically normal were randomly assigned to vaccine or placebo groups and divided into short-term duration of immunity (study #1) and long-term duration of immunity (study #2) studies. *S. neurona* sporocysts used for the challenge were generated in the opossum/raccoon cycle isolate SN 37-R. Study #1 horses received an initial vaccination and a booster, and were challenged 34 days post second vaccination. Study #2 horses received a vaccination and two boosters and were challenged 139 days post third vaccination. All horses in study #1 developed neurologic signs (n = 30) and there was no difference between the vaccinates and controls (P = 0.7683). All but four horses in study #2 developed detectable neurologic deficits. The neurologic signs, although not statistically significant, were worse in the vaccinated horses (P = 0.1559). In these two studies, vaccination with the *S. neurona* vaccine failed to prevent development of clinical neurologic deficits.

1. Introduction

Equine protozoal myeloencephalitis (EPM) is still one of the most important neurologic diseases of the horse (Dubey et al., 2001). According to estimates established by the National Animal Health Monitoring System, approximately 14 cases occur per 10000 horses per year (NAHMS, 2001). Unfortunately, treatment results in improvement in clinical signs only 70–75% of the time, relapses are common and many horses end up with residual deficits (MacKay et al., 2000; Saville et al., 2000a,b; Dubey et al., 2001). Therefore, the emphasis should be on prevention of the disease rather than on treatment. However, prevention of this disease has been limited to control of wildlife on the premises and prevention of the horses' feed from contamination with *S. neurona* sporocysts excreted by opossums (Saville et al., 2000a,b). Because approximately 50% of all horses in some areas of the USA are exposed to this organism, it is obviously difficult to prevent contamination (MacKay, 1997).

In 2000, Fort Dodge Animal Health (Fort Dodge, IA, USA) released a *S. neurona* killed vaccine for intramuscular use in horses that had been on a conditional license for the previous four years. Initially, the evaluation of the efficacy of this vaccine was limited in scope due to the lack of post vaccination challenge (Marsh et al., 2004; Witonsky et al., 2004). However, the development of an equine model of EPM (Saville et al., 2001; Sofaly et al., 2002; Saville et al., 2004) which had been further improved served as a suitable system to determine the efficacy of this vaccine. The purpose of the current study was to test the *S. neurona* vaccine to determine whether it would prevent the presence of clinical signs in experimentally challenged horses.

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2. Materials and methods

This research occurred from September of 2003 through May of 2004. The study was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Fort Dodge Animal Health under the auspices of the USDA.

2.1. Collection of S. neurona sporocysts and preparation of horse inoculum

Sarcocystis neurona sporocysts were obtained from laboratory-raised opossums fed tissues of raccoons that were fed sporocysts (isolate SN 37-R) as described (Stanek et al., 2002; Sofaly et al., 2002). The second passage of this isolate was used for the present study. For this, raccoons were euthanized 74 days after feeding sporocysts and their tissues were fed to four laboratory-raised opossums. The opossums were euthanized and sporocysts were collected from their intestines as described (Dubey and Hamir, 2000). Sporocysts were stored at 4 °C for 53 days (study #1) and 179 days (study #2) from the time of collection from opossums to feeding horses. Sporocysts were bioassayed through interferon gamma gene knockout (KO) mice at the time of horse inoculations to ensure viability as previously reported (Saville et al., 2001).

2.2. Experimental infection of horses

Draft and draft-crossed foals from an area of Saskatchewan, Canada were screened for antibodies to *S. neurona* using Western blot analysis (Dubey et al., 2003). Horses were obtained from this region because of the absence of opossum *(Didelphis virginiauus),* the only known definitive host for *S. neurona* in North America. Just prior to enrollment in the study, serum and cerebro spinal fluid (CSF) were collected as previously reported (Green et al., 1992). A total of 70 foals that were negative for *S. neurona* antibodies and were clinically normal were enrolled in this study and randomly assigned into a short-term duration of immunity study (study #1) and a long-term duration of immunity study (study #2). The horses in both studies were randomly assigned to receive either vaccination or a placeboon the day of selection at the horse farm in Saskatchewan using a random numbers table in Microsoft Excel. The placebo consisted of inactivated equine dermal cell line stock, Eagles Earles Media, Metastim (Fort Dodge Animal Health) as an adjuvant and preservatives including: polymixin B, neomycin, and thimerosal.

Foals used in study #1 (Horse Nos. 1–35), the short-term duration of immunity study, received a vaccine (n = 17) or placebo (n = 18) on day-1 and received a booster on day-28. They were serum sampled on days 14 and 28 for detection of *S. neurona* antibodies. They were also serum sampled at 14 days after the booster (day-35) to evaluate *S. neurona* antibodies pre- and post-immunization samples. Study #1 foals were shipped to Fort Dodge, Iowa (FDAH) 34 days following the booster vaccination (day-62). These foals were 4–5 months old. Upon arrival at the study site at FDAH, all horses were examined physically and neurologically by a blinded observer. Samples collected following the neurologic examination included blood using serum separator tubes and whole blood for complete blood count using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. Following sedation (Sedazine® and Torbugesic®; Fort Dodge Animal Health, Fort Dodge, IA,USA), CSF was collected from each horse via the lumbosacral space as reported previously (Green et al., 1992). After CSF collection, all horses were inoculated with 1.5×10^6 *S. neurona* sporocysts by nasogastric intubation.

Foals used in study #2 (Horse Nos. 36–70), the long-term duration of immunity study, received a vaccine (n = 17) or placebo (n = 18) on day-1 and received boosters on day-21 and again on day-28. They were serum sampled for *S. neurona* antibody on day-21, day-35, day-42, and day-49 to compare antibody responses both pre- and post-immunization. Study #2 horses were shipped 139 days following their second booster vaccination (day-167) to FDAH. These horses were approximately 1 year of age. All procedures were followed as previously described for study #1. Following CSF collection, this group of horses received 3.0×10^6 *S. neurona* sporocysts by nasogastric intubation.

At the FDAH facility, horses were housed in an isolation barn with concrete floors and rubber mats for bedding. They were fed heat-treated feed (Buckeye Feeds, Dalton, OH, USA) to kill any sporocysts that might be present. Water was provided *ad libitum*. The stalls in the isolation barn were open steel panels. All horses were examined daily and findings were recorded by the persons at FDAH. Weekly serum samples were collected at FDAH for evaluation of seroconversion to *S. neurona* antigens to compare to pre-challenge and post-challenge samples. Neurologic examinations were performed at 7, 14, 21, and 28 days after inoculation (DAI) at FDAH by the same masked observer.

2.3. Statistical analysis

All horses were examined by a masked examiner who recorded a grade for each limb from 0 to 5 of 5 for weakness, spasticity, and ataxia. An overall scoring system to establish a neurologic grade for each horse was determined based on the addition of points scored for each limb. This scoring system was determined based on the distribution of scores for all horses at each time point. Horses that scored 1–3 points had an overall score of 0.5 of 5; horses that scored > 3–6 points had an overall score of 1 of 5; horses that scored > 6–9 points had an overall score of 1.5 of 5; horses that scored > 9–12 points had an overall score of 2 of 5; horses that scored > 12–15 points had an overall score of 2.5 of 5; horses that scored > 15–18 points had an overall score of 3 of 5; and horses that scored > 18–21 points had an overall score of 3.5 of 5. The overall scores for each horse were determined by each group, using the group of horses that received the placebo as a control group. The mean scores were compared by group using Wilcoxon Rank Sum test (SAS Institute, Inc, Cary, North Carolina, version 8.2) with a significance level of $P < 0.05$.

3. Results

3.1. Experimental horse results

3.1.1. Physical examination of horses from study #1

On the day of arrival at FDAH, some horses had mucoid nasal and ocular discharge. Five horses had to be eliminated, four due to neurologic deficits (Nos. 5, 14, 29, 32) and one due to handling problems (No. 25). One horse developed fever, diarrhea, and colic and was euthanized at 17 DAI after two days of therapy. Nine other horses developed fevers on 28 DAI, and four were reluctant to walk. The four horses reluctant to walk were suspected of having laminitis and were treated with non-steroidal inflammatory medications.

3.1.2. Neurologic examination of horses from study #1

All horses in both the vaccinated and placebo groups developed neurologic deficits. The vaccinated horses neurologic scores ranged from 0.5 to 3.5 of 5 (Table 1). The range of neurologic scores in the placebo group was 0.5–4 of 5 (Table 1). Neurologic deficits were seen in the vaccinated horses at 7 DAI in all but 1 horse. In the placebo group, neurologic deficits were detected at 7 DAI in all but two horses.

3.1.3. Serum and CSF examination in horses from study 1

Of the 15 vaccinated horses, 4 of 15 were serum positive for antibodies to *S. neurona* after 1 dose and 14/15 serum positive after the second dose. On the day of challenge, 14 of 15 were serum positive, and all 30 study horses were CSF negative. All 15 placebo horses were negative in serum at day of challenge. The 14 of 15 vaccinated horses were the only ones positive in serum at 7 days after inoculation (DAI). All 15 vaccinated horses were serum positive at 14 DAI while only 2 of 15 placebo horses were serum positive at that time. At 21 DAI, all vaccinated horses were serum positive, but only 10 of 15 placebo horses were positive. On the last day of the study, 28 DAI, all vaccinated horses

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