Vaccination of horses with Lyme vaccines for dogs induces short-lasting antibody responses

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1. Introduction

Lyme disease is induced by Borrelia burgdorferi, a spirochete that is transmitted to humans, dogs, horses, and other mammals by ixodes ticks [1–3]. Controversial opinions exist about the occurrence of Lyme disease in horses. Clinical signs associated with Lyme disease include lameness and behavioral changes which are rather non-specific and can have different causes. Experimental infection inducing the disease in horses has not yet been reported. High seroprevalence in clinically healthy horses in endemic areas further adds to the controversy [2]. Nevertheless, accumulating evidence supports Lyme disease in infected with B. burgdorferi. Most commonly reported clinical signs are sporadic lameness and diverse orthopedic problems, often associated with shifting or multiple leg lameness, muscle tenderness, hyperesthesia, chronically poor performance, swollen joints, or arthritis [4,5]. Less frequently described are cases of Borrelia-associated pseudolymphoma [6], uveitis [7], and neurologic signs including ataxia, depression, head tilt and encephalitis [8–10].

The diagnosis of Lyme disease in horses is based on clinical signs compatible with the disease in the absence of other causes, potential exposure to ticks, and positive serological testing for antibodies to B. burgdorferi [5]. Response to treatment alone is not sufficient to confirm diagnosis due to the anti-inflammatory effects of commonly used antibiotics [11], but follow-up antibody testing post-treatment and decline of B. burgdorferi-specific antibodies can support the diagnosis [5]. Antibody testing for B. burgdorferi has improved in recent years, with a quantitative Lyme Multiplex assay becoming available. The Lyme Multiplex assay can distinguish between early and chronic infection stages, via antibodies against outer surface protein C (OspC) and OspF, as well as measure responses to vaccination by quantification of OspA antibodies [12,13]. In addition, OspF of B. burgdorferi is the preferred antigen for confirmation of chronic infection in horses because antibody
responses to OspF are more widely distributed than those to C6, thereby allowing improved quantification [12].

Currently, prevention of Lyme disease is primarily focused on decreasing risk of exposure to ticks. However, vector control methods are typically not sufficient on their own, leading veterinarians to utilize Lyme vaccines [5]. Approved Lyme vaccines for horses are not currently available, despite evidence that OspA antibodies may protect horses from infection [14]. Several Lyme vaccines are currently approved in the US for use in dogs, some of which have been given off-label to horses. Most of the canine vaccines are whole-bacterin vaccines. One is composed of recombinant OspA only, which is known to be self-adjuvating [15] and was shown to be bacterin vaccines. One is composed of recombinant OspA only, given off-label to horses. Most of the canine vaccines are whole-rntly approved in the US for use in dogs, some of which have been safe and efficacious in dogs [16]. The serologic response to successful Lyme vaccination is typically characterized by high values for antibodies to the OspA antigen. High amounts of OspA are expressed when B. burgdorferi is cultured in vitro [17, 18], leading to bacterin-based vaccines containing this immunogenic protein. Anti-OspA antibodies have been shown to protect from infection by inhibiting transmission of B. burgdorferi from the tick vector to mammalian hosts, as frequently described in humans [19, 20], laboratory rodents [21, 22], dogs [23, 24], and also in horses [14].

The OspA protein of B. burgdorferi is essential for colonization and survival of the bacteria within the tick midgut [25, 26]. During transmission to the mammalian host, OspA becomes downregulated in the bacteria, followed by swift up-regulation of OspC [27]. Bacterial strains that produce elevated levels of OspC in culture have been developed for use in vaccines [28]. The OspC protein plays a role in bacterial invasion of the tick salivary gland and transmission of bacteria is assumed to be partially inhibited if the tick blood meal contains anti-OspC antibodies [29, 30]. There is some evidence that antibodies against OspC may also have bacterioidal effects [31, 32], but to the author’s knowledge, there is no direct evidence that anti-OspC antibodies offer protection from B. burgdorferi infection in horses.

The goal of this work was to evaluate the serologic response in horses to vaccination with Lyme vaccines for dogs. We first evaluated diagnostic samples submitted by veterinarians in the field, and then investigated antibody responses induced by these vaccines after a series of experimental vaccinations in a controlled herd of horses.

2. Materials and methods

2.1. Horses and horse environment

2.1.1. Diagnostic samples

Serum samples from horses at multiple locations in the US were submitted by veterinarians for Lyme Multiplex testing to the Animal Health Diagnostic Center (AHDC) at Cornell University between July 2011 and July 2014. Additional information on vaccination status was obtained from the respective equine practice when the submission form indicated ‘vaccination against Lyme disease’, including date and manufacturer of the most recent vaccine administered. Horses were included in this study if the most recent vaccination was at least one and less than ten months prior to sample submission. This resulted in 65 samples from horses vaccinated with Duramune Lyme® (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), 105 samples from horses vaccinated with Recombitek® Lyme (Merial Inc., Duluth, GA). Pre-vaccination serology and infection history was not available for these animals.

2.1.2. Horses for experimental vaccination trials

A total of 47 Icelandic horses of the specific-pathogen-free herd at Cornell University were enrolled in this study [33, 34]. All horses were kept at this facility before and during the experimental vaccination study. Lyme disease has not been reported in Iceland [35]. At the time of importation, horses were thus considered Lyme-free and immunologically naïve to B. burgdorferi. After release from importation quarantine, horses were kept on pasture all year long. The pasture area was surrounded by woods and frequently visited by wildlife. Grass hay was fed ad libitum. Horses were annually vaccinated against rabies, tetanus, West Nile Virus, Eastern and Western Encephalitis, and were regularly dewormed. Horses were clinically healthy throughout the studies. A summary of age, gender and vaccination groups of the horses in the four experimental vaccine trials is shown in Table 1.

2.2. Experimental vaccine approach and blood sample collection

Unless stated otherwise, vaccinations were performed intramuscularly (IM) into the pectoral muscle using 20-gauge needles. Blood samples for serum collection were obtained by jugular venipuncture with a vacutainer collection system without coagulant. If horses were vaccinated on a specific day, blood samples were taken directly before vaccination. Serum was harvested from clotted blood by centrifugation at 700g for 10 min and was stored at –20 °C until serological analysis. All animal procedures for this study were carried out in accordance with the recommendation in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. The animal protocol was approved by the Institutional Animal Care and Use Committee, Cornell University (protocol #2011-0011).

2.2.1. Vaccination trial #1 – vaccine comparison

Lyme naïve horses in the 1st experimental Lyme vaccination trial received their first Lyme vaccine dose in the quarantine barn immediately before they were released to the pasture in April 2013. Initially, we compared the serologic response of B. burgdorferi naïve horses to the three US commercial canine Lyme vaccines available in 2013 (Supplementary Table 1): Duramune Lyme® (lot#: 522108A), Recombitek® Lyme (lot#: 42180A), and Nobivac® Lyme (lot#: 02161126). Seven horses were assigned to each group (Table 1). One animal from the Nobivac® Lyme group had to be removed from the trial due to reasons unrelated to this study and was not included in the analysis. Animals received a 1 mL dose of vaccine on days 0, 25, and 108. An additional 9 horses were not vaccinated and monitored as a control group.

2.2.2. Vaccination trial #2 – dose comparison

Some practitioners double the dose of the dog vaccine when used in horses. Trial 2 evaluated the impact of dose on the magnitude and duration of antibody responses to vaccination. Yearlings and two-year-old horses received their first Lyme vaccination in April 2014. Prior to this vaccination, they were kept on pasture with potential exposure to B. burgdorferi infected ticks for one or two years, respectively. Nobivac® Lyme vaccine (lot#: 02161130 for the initial vaccination [day 0]; lot#: 02161131 for the boost [day 24]) was used for vaccination. The 27 foals were randomly assigned into two age-matched groups. Foals received 1 mL (n = 13) or 2 mL (n = 14) of vaccine per injection. An additional 5 horses were vaccinated and monitored as a control group.

2.2.3. Vaccination trial #3 – impact of previous Lyme vaccination

In 2015, one year following vaccination trial #2, 18 of the horses were vaccinated with 1 mL Recombitek® Lyme (lot#: 42198) on day 0 and day 28 to analyze if previous vaccination impacted magnitude and duration of serologic responses to vaccination. Another 9 horses vaccinated with Nobivac® Lyme the previous year were not vaccinated and monitored as a control group.
2.2.4. Vaccination trial #4 – influence of injection route

Finally, the influence of injection site, subcutaneous (SC) vs. IM, on the immune response was compared in previously vaccinated horses. In 2016, a single 1 ml dose of Recombitek Lyme (lot #: 42203) was either injected IM into the pectoral muscle (n = 7), or SC into the subcutis on top of the pectoral muscle (n = 7) on day 0. All vaccinated horses received two doses of 1 ml Recombitek Lyme IM in the previous year. Eight horses from the previous year’s control group continued to be non-vaccinated and monitored.

2.3. Tick monitoring, collection and PCR

The horses were examined for reasons unrelated to this study (allergy scoring) every week in spring, summer and fall, and 2–3 times per month in the winter. They were touched and examined at the face, submandibular space, chest, belly, and other body areas during the allergy scoring. As soon as the first tick was found, the horses were systematically checked for ticks by thoroughly running hands around the ears, nose, chin, along the submandibular region, down the neck, along the chest, and between the front legs and the girth area. Although single ticks may have been missed occasionally by this procedure, we have examined the horses regularly throughout the years of the experimental Lyme vaccination studies described here to identify the major tick activity times and to estimate the percentages of *B. burgdorferi* infected ticks in their environment. The times when ticks were collected from the horses were in spring (April/May) and fall (October/November). Ticks collected from horses were stored in 70% ethanol at 4 °C until PCR analysis. All collected ticks identified as *Ixodes scapularis* were analyzed for *B. burgdorferi* DNA by PCR at the AHDC, Cornell University.

2.4. Serological detection of antibodies against *B. burgdorferi*

All serum samples were measured for antibodies to OspA, OspC and OspF by the Lyme Multiplex assay at the AHDC, Cornell University, as described previously [13].

2.5. Statistical analysis

The comparison of antibody values between the different vaccine groups was performed using repeated-measures ANOVA with Tukey’s multiple comparison tests and the GraphPad Prism program version 6. P-values (p < 0.05) were considered significant.

### Table 1

Experimental vaccination trials. Overview about the experimental vaccination trials of horses with Lyme vaccines for dogs.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vaccine</th>
<th>Doses</th>
<th>Vol per dose (ml)</th>
<th>Route</th>
<th>Horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Recombitek Lyme</td>
<td>3</td>
<td>1 ml</td>
<td>i.m.</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Nobivac Lyme</td>
<td>6</td>
<td>1 ml</td>
<td>i.m.</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Duramune Lyme</td>
<td>7</td>
<td>1 ml</td>
<td>i.m.</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Nobivac Lyme</td>
<td>2</td>
<td>1 ml</td>
<td>i.m.</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Duramune Lyme</td>
<td>2</td>
<td>2 ml</td>
<td>i.m.</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>Recombitek Lyme</td>
<td>2</td>
<td>1 ml</td>
<td>i.m.</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Not vaccinated</td>
<td>9</td>
<td>2–3</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Recombitek Lyme</td>
<td>3</td>
<td>1 ml</td>
<td>s.c.</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Not vaccinated</td>
<td>8</td>
<td>3–4</td>
<td>i.m.</td>
<td>17</td>
</tr>
</tbody>
</table>

### Table 2

Horse diagnostic submissions. Serum antibodies against *B. burgdorferi* OspA (MF) of horses by months after vaccination with commercial Lyme vaccines for dogs. MF > 2000 is the Equine Lyme Multiplex assay positive cut-off value for antibodies against OspA.

<table>
<thead>
<tr>
<th>Months post vaccination</th>
<th>Whole bacterin vaccine (Duramune Lyme)</th>
<th>Recombinant OspA vaccine (Recombitek Lyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median anti-OspA value (range)</td>
<td>Samples with anti-OspA &gt; 2000</td>
</tr>
<tr>
<td>1–3</td>
<td>1563 (453–10733)</td>
<td>9/18</td>
</tr>
<tr>
<td>4–6</td>
<td>469 (54–4685)</td>
<td>4/30</td>
</tr>
<tr>
<td>7–9</td>
<td>514 (97–5864)</td>
<td>1/17</td>
</tr>
</tbody>
</table>

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values > 2000 MFI 7–9 months post-vaccination (Supplementary Table 2). OspC and OspF antibody values were not analyzed in this sample set based on the unknown exposure and treatment status of the horses. The OspA antibody results corresponded to data from vaccination trials using rOspA expressed from E. coli showing that dogs maintained protective OspA antibodies 6-months after vaccination [37]. In contrast, vaccinated ponies (n = 7) developed OspA antibodies which began to decline prior to a booster dose administered on day 82 [14].

The differences in magnitude and duration of the OspA antibody response after Lyme vaccination observed here in diagnostic sample submissions from horses (Table 2) and dogs (Supplementary Table 2) can have several reasons: (1) the vaccine history and OspA antibody values prior to the last vaccination was unknown for these horses and dogs. Dogs may have obtained higher overall numbers of annual Lyme vaccinations than horses, resulting in the observed differences between the species; (2) the optimal vaccination dose for horses is not yet established. The vaccine dose was not available and may have been suboptimal for horses included in this dataset; (3) many other confounders, such as age, breed, risk of infection with B. burgdorferi or previous infection/treatment data were not available for the animals contributing to these samples and have the potential to influence the response to vaccination; (4) horses may respond to the available Lyme vaccines for dogs with a broader range and sometimes lower immune response than dogs. This could be due to differences in major histocompatibility complexes and antigen presentation of the OspA antigen in these two species.

In summary, the analysis of diagnostic samples from vaccinated horses suggested that Lyme vaccines for dogs can result in short-lasting OspA antibody responses of varying magnitude in horses. We initiated a series of experimental vaccination trials in a controlled group of horses to further investigate antibody induction by available Lyme vaccines.

3.2. Antibody response in B. Burgdorferi naïve horses

A first experimental vaccine trial tested three available Lyme vaccines for dogs in horses without previous exposure to B. burgdorferi. During this trial in 2013, horses had a low-moderate risk of exposure to B. burgdorferi with 4.3% PCR positive ticks collected from the horses (Supplementary Table 3). After an initial vaccination followed by one boost, horses developed overall low OspA antibody values, with mean OspA antibody values dropping below 2000 MFI in less than 16 weeks, independent of the vaccine used (Fig. 1A). Only 3/7, 4/7 and 5/6 horses, respectively, developed OspA antibody values > 2000 MFI following the first booster dose of Duramune Lyme®, Recombitek® Lyme, and Nobivac® Lyme. A trend towards higher OspA antibody values was observed in response to Nobivac® Lyme vaccination (Fig. 1A). Horses vaccinated with Nobivac® Lyme also developed significantly higher OspC antibody values following both booster vaccinations on days 25 and 108 (Fig. 1B). Horses from the other two groups did not mount an OspC antibody response. The third vaccination on day 108 was performed because of overall low antibody responses to the first two vaccine doses. It has been reported that a third vaccination dose in dogs resulted in significantly increased OspA antibodies values compared to the tradition two dose series [24]. In the horses vaccinated here, the OspA antibody response was of similar magnitude after the 2nd and 3rd vaccine administrations. Only the mean OspC antibody response was elevated following the 3rd dose of Nobivac® Lyme vaccine (Fig. 1B). Following the third dose, only Nobivac® Lyme vaccinated horses trended towards longer duration OspA immune response (Fig. 1A). However, by 24 weeks following the 3rd dose, mean OspA antibody values dropped again below 2000 MFI.

Elevated OspF antibody values, indicative of infection with B. burgdorferi, were not detected for the duration of the vaccination trial, with the exception of two horses in the Recombitek® Lyme vaccine group on days 61–108 and day 137 post-vaccination, respectively (Fig. 1C). These OspF responses were likely related to subclinical infection with B. burgdorferi.

Overall, the OspA antibody responses after experimental vaccination of horses with commercial Lyme vaccines for dogs confirmed that these vaccines induce short-lasting antibody responses.
in *B. burgdorferi* naïve horses. OspA antibody responses were low to almost not detectable in individual horses, while others mounted OspA antibodies as expected after vaccination. It should be noted that all horses in the experimental Lyme vaccine trials were Icelandic horses. This could be seen as strength because it removes breed as a possible confounding difference. However, it could also be speculated that these horses represent a group of individuals that is genetically similar and that the unique breed of the horse may have contributed to the low vaccine response observe here. Based on our previous observations, it is rather unlikely that the OspA antibody responses of these horses were reduced because of their breed. Icelandic horses responded to equine herpesvirus type I (EHV-1) infection [34] and EHV or West Nile virus vaccination with high antibody responses similar to other breeds [33,38]. Nevertheless, one possible explanation for the low OspA response in these horses is that the Lyme vaccine dose of 1 mL used in this trial was optimized for dogs and is potentially too low for horses.

### 3.3. Influence of vaccine dose on antibody responses

Of the three vaccines tested, Nobivac® Lyme produced the most notable antibody response in horses. This vaccine was tested again in previously non-vaccinated horses to investigate whether doubling of the vaccine dose increases the antibody response. Both OspA antibody values (Fig. 2A) and OspC antibody values (Fig. 2B) were significantly increased in animals receiving 2 mL instead of 1 mL doses per vaccination (p < 0.01 and p < 0.0001, respectively). Nevertheless, the duration of antibody response after vaccination with both doses was again short and comparable to that observed in trial 1 with this vaccine. A total of 12/14 horses that received 2 mL doses developed OspA antibody values > 2000 MFI at 8-weeks post-vaccination, compared to only 8/13 horses that received 1 mL doses. By 18-weeks post-vaccination, 8/14 horses that received the 2 mL doses maintained OspA antibody values > 2000 MFI, compared to only 1/13 that received the 1 mL doses. By 7 months post-vaccination, only 2/14 horses that received the 2 mL doses maintained OspA antibody values > 2000 MFI. These results confirmed that doubling the dose of the canine Lyme vaccine for horses resulted in an enhanced magnitude but still short-term duration of antibody responses.

Unlike the first vaccination trial, where horses were naïve to *B. burgdorferi* exposure, the animals in this trial experienced 1–2 years in an endemic environment, and >21% of the 55 ticks collected from horses during this trial were PCR positive for *B. burgdorferi* DNA (Supplementary Table 3). Interestingly, unlike in naïve horses, where only OspA and OspC antibody values increased in response to Nobivac® Lyme vaccine, in this trial, OspF antibody values were also elevated following the booster dose (Fig. 2C). Experimental infection studies in dogs revealed that OspF antibodies can be detected with the Lyme Multiplex assay by 5 weeks post infection and afterwards [36]. A similar experimental approach to identify the onset of OspF antibodies in infected horses has not yet been performed, but it can be expected that these will be detectable at approximately the same time as in dogs. Although natural infection could not be completely ruled-out in our vaccinated horses developing OspF antibodies, no ticks were found on horses in the herd in the months preceding the initial and booster vaccination. In addition, a group of non-vaccinated horses did not develop OspF antibody value increases in the same time-period (Supplementary Fig. 1B). These findings suggest that the whole-bacterin-based Nobivac® Lyme vaccine, which contains many antigens of *B. burgdorferi*, can induce low and short-lasting antibodies against OspF and other antigens, including C6 (data not shown). Previous studies refuting this claim were performed in *B. burgdorferi* naïve dogs [39]. It is known that cultured killed bacteria express all of these proteins and this fact has been used for many years for Lyme Western blot diagnostics to determine serological responses to various antigens of *B. burgdorferi* [40,41]. Consequently, vaccination with whole-bacterin Lyme vaccines can interfere with the ability to diagnose both acute and chronic infections, unlike vaccination with the OspA-based Recombitek® Lyme vaccine.

### 3.4. Effect of previous vaccination on OspA antibody duration

Horses vaccinated with Nobivac® Lyme in the previous year were vaccinated with two doses of Recombitek® Lyme to evaluate the effect of previous vaccination on the OspA antibody response.
The Recombitek® Lyme vaccination resulted in an earlier onset of OspA antibodies, with 15/18 horses developing OspA antibody values > 2000 MFI prior to the booster dose (Fig. 3A). Additionally, there was a trend towards increased longevity, with 7/18 horses maintaining OspA antibody values > 2000 MFI at >18 weeks post-vaccination (Fig. 3A). Compared to Recombitek® Lyme vaccination in naive horses, both intensity and longevity of the response were increased (Supplementary Table 4). However, by 24 weeks post-vaccination, OspA antibody values were again < 2000 MFI in all horses (Fig. 3A).

Antibodies against OspC were not observed in this trial (Fig. 3B). However, 2 out of 9 horses in the non-vaccinated control group developed elevated OspF antibody values indicative of infection with B. burgdorferi in late fall after a period of potential exposure to infected ticks (Fig. 3C). None of the horses in the vaccinated group showed increased OspF antibodies, suggesting that OspA antibodies mounted in the vaccinated group resulted in protection from infection.

3.5. Influence of injection route on antibody response

A single dose annual booster vaccination with Recombitek® Lyme in the following year produced a short-lived OspA response in all animals, regardless of SC or IM injection route. Only 2/14 horses maintained OspA antibody values > 2000 MFI at 16 weeks post-vaccination (Fig. 4A). Four out of 7 and 5/7 animals, respectively, developed OspA antibody values > 2000 MFI 19 days after SC and IM injection. The magnitude of the peak OspA antibody response was similar after SC or IM vaccination (Fig. 4A). OspC or OspF antibodies were not elevated during the trial (Fig. 4B/C). SC versus IM injection route were previously reported to impact the immune response to a vaccine [42]. More frequently, as also seen here, these injection routes resulted in equivalent immune response to vaccination [43–45].

The horses vaccinated here had received two doses of the same vaccine, Recombitek® Lyme, in the previous year. The annual boost given during the injection route trial resulted in a faster declining OspA antibody response of lower magnitude (Fig. 4A) compared to the OspA response observed in the previous year (Fig. 3A). This suggested that annual revaccination intervals may not be sufficient for many horses to maintain OspA antibodies for more than 3–4 months post vaccination.

4. Conclusions

Lyme vaccination has the potential to protect horses from infection with B. burgdorferi and is especially desirable in
Lyme-endemic areas, where animals can be regularly re-infected. OspA antibodies have been identified as correlates for protection from infection with *B. burgdorferi* in many species, including horses [14,19–24]. Our current results highlight that Lyme vaccines for dogs induce OspA antibodies in horses only transiently and inconsistently. However, the data presented here also indicate that increasing the vaccination dose for horses can enhance the OspA antibody response, and adding another booster vaccination can extend the duration of the OspA antibody response. As long as optimal vaccination doses and intervals are not established for horses, currently available Lyme vaccines for dogs can be suboptimal in inducing OspA antibodies. Although individual horses may respond to vaccination with high OspA antibody responses, many horses mount very low OspA antibody values that are unlikely to offer full protection over the course of a year.

Additionally, this work revealed that vaccination with whole-bacterin vaccines may interfere with subsequent diagnosis. Particularly in animals with likelihood for previous exposure to *B. burgdorferi*, whole-bacterin Lyme vaccine may boost pre-existing OspC, OspF and C6 antibody responses. This interference with Lyme interpretation can be avoided by using the OspA-based vaccine.

To the authors’ knowledge, major adverse reactions to Lyme vaccination in horses have not been reported and were also not observed during the experimental vaccination trial performed here. Based on our current data, OspA antibody responses after Lyme vaccination are short-lasting. It can thus be recommended to vaccinate horses in close proximity to tick season, approximately 4 weeks before ticks are typically abundant in the area. Based on our current data, OspA antibody responses after Lyme vaccination are short-lasting. It can thus be recommended to vaccinate horses in close proximity to tick season, approximately 4 weeks before ticks are typically abundant in the area. Based on our current data, OspA antibody responses after Lyme vaccination are short-lasting. It can thus be recommended to vaccinate horses in close proximity to tick season, approximately 4 weeks before ticks are typically abundant in the area.

5. Contribution of authors

BW planned and performed the vaccination approach and performed the data analysis. CG drafted the manuscript with contributions from BW and assisted with data analysis. SA and JR were instrumental in gathering diagnostic submission data. AG directed the tick analysis and *B. burgdorferi* PCR. All authors contributed to the preparation of the article and approved the final version prior to submission.

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7. Conflict of interest

BW is the inventor on a patent entitled ‘Methods for Diagnosing Lyme Disease’, Patent #: US 8,946,393 B2 that uses technology described in this manuscript.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.06.052.

References


