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Short communication

A study with a commercial vaccine against Lyme borreliosis in horses using two different vaccination schedules: Characterization of the humoral immune response

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ABSTRACT

A total of 143 horses were included in a study to test a commercial vaccine against Lyme borreliosis. The vaccine contained three different antigens (outer surface protein A, OspA) to prevent the infection with spirochetes - *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. Horses in Group A (49 animals) received two vaccinations on days 0 and 14 and a booster on day 365, whereas 50 horses in Group B received an additional booster vaccination on day 180. Group C (44 animals) was not immunized. Total antibody levels and specific OspA antibody responses were assessed quantitatively and qualitatively in two-month intervals over 13-month period. Vaccinees in Groups A and B developed high OspA antibodies levels, whereas horses in Group C did not show specific antibody responses. The additional vaccination applied in Group B enhanced the specific OspA antibody response significantly and prevented its rapid decline.

1. Introduction

Spirochetes of the *B. burgdorferi* sensu lato (*Bbsl*) complex can cause a multisystemic disease, known as Lyme borreliosis (LB), in humans and animals [1]. Within the European realm, there are at least three different important genospecies: *B. burgdorferi* sensu stricto (*Bbss*), *B. afzelii* and *B. garinii* [2]. The occurrence of LB in horses is discussed extremely controversial. Nevertheless, there are numerous clinical case studies providing evidence that an extensive amount of clinical changes in horses such as general disturbances and lameness exist [3–5]. In this context, the infections with *Bbsl* can be confirmed indirectly by the detection of *Bbsl*-specific antibodies. A two-tiered test, which consists of a specific antibody ELISA and an immunoblot is deemed the gold standard for LB diagnosis [6].

Vaccines are an effective measure to prevent disease. The mechanism of action in the case of LB vaccines is based on an induction of antibodies against the outer surface protein A (OspA), which have been shown to inhibit transmission of spirochetes from ticks to mammalian hosts. The presence of high levels of OspA-specific antibodies is necessary for an effective protection against infection as shown for humans [7], dogs [8] and horses [9]. Since June 2015 a LB vaccine for horses (EquiLyme®) is available. It is supposed to induce OspA-specific antibodies against *Bbss*, *B. afzelii* and *B. garinii*. In order to induce an protective humoral immune response, the immunization has to be applied regularly [10]. No data exist concerning the antibody kinetics after vaccination with EquiLyme®. With regard to diversity of borrelia species it needs to be considered that OspA antibodies may show very low cross-protection among LB species [11], because these antibodies bind precisely to a specific epitope on their OspA variants [10]. The aim of this project was to study the horses' immune response after immunization with EquiLyme® and comparing two different vaccination strategies. Species-specific OspA antibody responses against *Bbss*, *B. garinii* and *B. afzelii* were charcterized.

2. Materials and methods

In total, 194 randomly selected privately owned horses were examined clinically and tested serologically for possible borrelia infections using a kinetic enzyme-linked immunosorbent assay (KELA) and a line immunoblot assay (LIA). A subpopulation of 143 healthy and seronegative or weakly positive individuals (<300 KELA units) were included in this study (Supplementary Fig. 2). At the end of the 13-month observation period, 130 horses

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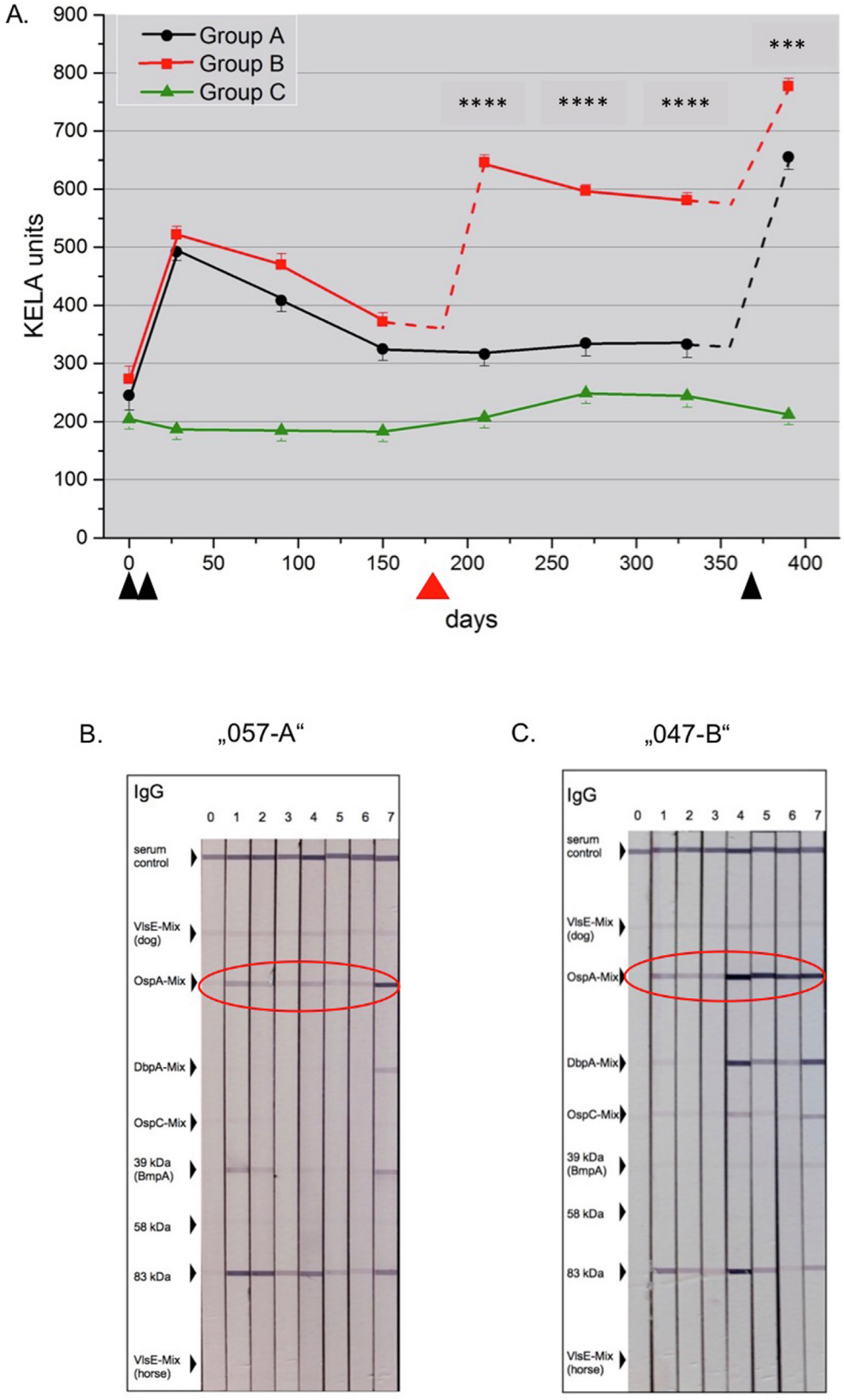

Fig. 1. A. Antibody levels induced with EquiLyme® detected by KELA. Horses in group A (vaccinated on days 0 and 14 and 365) are shown with round symbols; horses in group B (vaccinated on days 0, 14, 180 and 365) are shown with quadrangular symbols; horses in group C (not vaccinated) are shown with triangular symbols. IgG antibody detection was accomplished with bacterial lysates produced with *Bbss* N40 enriched with rOspA of *Bbss* (Recombitek® Lyme). Arrows indicate to the time points of vaccination. The dash lines indicate the projected antibody kinetics of group A and B. ▲ = additional vaccination in group B. ***$p < 0.001$ **** $p < 0.0001$. Fig. 1B,C. Borrelia Veterinary plus OspA LINE of the serum samples collected from horses "057-A" (group A) and "047-B" (group B). The first sample was drawn on day 0 (pre-vaccination); lane 1 shows results 28 days after the first vaccination; samples shown in lanes 2–7 (days 90–390) were collected in two-months intervals; lane 4 of "047-A" shows the results 30 days after an additional booster on day 180; the sample on lane 7 (day 390) was collected 28 days after booster vaccination was applied on day 365 +/−3 days.

were available for complete analysis. Horses in Group A (49 animals) were immunized with an 1-ml vaccine dose on days 0, 14 and 365 ± 3. Horses in Group B (50 animals) received immunizations on days 0, 14, 180 and 365 ± 3. Horses in control Group C (44 animals) were not vaccinated. Four weeks after the final booster vaccination on day 365 ± 3, the last blood samples were collected (Supplementary Fig. 3). All procedures for this study were carried out in accordance to the guidelines approved by the Animal Welfare Committee of the Government of Upper Bavaria, Munich, Germany (Az. 55.2.1.54-2532.0-91-16) and the protocols adhered to the German "Tierschutzgesetz" (§ 8a Abs. 1 Nr. 3b TierSchG).

The vaccine EquiLyme® (Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany) was approved for its application in horses on June 16, 2015 by the Paul-Ehrlich-Institute (PEI) in Langen, Germany (Approval-/Reg-No.(AMG76): PEI. V.11736.01.1). One dose (1 ml) of the lysate vaccine (lot#: 476123A) contained antigens of *Bb*ss, *B. afzelii* and *B. garinii* in an inactivated form. Aluminum hydroxide served as an adjuvant. Immunizations were performed intramuscularly (IM) into the pectoral muscle using 22-gauge needles (0.70 × 32 mm). Blood samples for serum collection were collected by jugular venipuncture using 9-ml Serum-Monovettes Z-Gel, with corresponding multi-adapters (Sarstedt GmbH & Co. KG, Nümbrecht, Germany) and 1.20 mm × 40 mm needles (Henry Schein, Langen, Germany). Blood samples were stored at room temperature for at least 30 min; serum was harvested by centrifugation at 700 × *g* for 10 min and stored at −20 °C until used.

Total antibodies levels specific for LB organisms and OspA-specific antibody levels were measured with a kinetic ELISA (KELA) as described elsewhere [8,12,13]. Purified recombinant OspA (1.0 µg per well for strains *Bb*ss ZS7, *B. garinii* ZQ1 or *B. afzelii* PKo kindly provided by Prof. Dr. Kraiczy, Institute for Medical Microbiology and Infection Control, Frankfurt, Germany) were used to measure OspA-specific antibody levels over time. The Borrelia Veterinary plus OspA LINE (Sekisui Virotech GmbH, Rüsselsheim, Germany) is a LIA for a qualitative characterization of *Bb*sl-specific antibodies. Antibody detection with LIA strips was conducted according to the manufacturefs instructions.

Antibody levels for each horse were recorded, summarized according to the experimental groups and compared statistically. Calculations were done with R-cran (www.cran.r-project.org). For each animal the area under the curve (AUC) was calculated from the respective antibody level and the time, using the triangulation method (connecting two points with a direct line). The AUCs were compared between the three groups using one-way ANOVA and Tukeys HSD correction for the pairwise comparisons between the groups. A *p*-value of 0.05 was set as significance level.

3. Results

An increase of vaccine-induced specific antibodies was observed in Groups A and B by day 28 after the first immunization (Fig. 1A). On day 150, total antibodies in Groups A and B practically dropped back to the pre-vaccination level. Following booster vaccination on day 180, horses in Group B developed a significant ($p < 0.001$) rise in antibody levels compared to day 28, while antibody levels of horses in Groups A and C were below the detection limit. Due to the booster vaccination at the end of the study, horses in Groups A and B showed significantly elevated vaccination antibody levels ($p < 0.001$ compared to Group C). Horses in Group B developed significantly higher antibody levels after the additional four immunizations with an average increase by 122 KELA units when compared to Group A (Fig. 1A; $p < 0.001$).

Fig. 1B shows the qualitative antibody response of horse "057-A", representative for the horses in Group A. Before vaccination, no bands were visible (lane 0). When the regular immunization regimen consisting of two vaccine applications was applied, signals appeared for OspA, BmpA (39 kDa) and p83 (lane 1). While signals for OpsA waned considerably by day 150 (lane 3), a prominent signal at p83 was still detectable around day 210 after vaccination (lane 4). Due to the last booster immunization an additional band was visible for the DbpA-mix, besides the stronger signal for OspA, p39 and p83 (lane 7). Fig. 1C shows the vaccine-induced antibody response of horse "047-B", representative for horses in Group B. Signals are visible for p83 and OspA (lane1) during the early days of the observation period. Starting with day 90 (lane 2), signal intensity decreased continuously until day 150 (lane 3). The additional booster immunization on day 180 intensified signals for OspA and p83 and bands for DbpA and OspC became visible (lane 4). Subsequently, signal intensities for p83, DbpA and OspC decreased (lanes 5–6), whereas the last booster immunization on day 365 produced intense bands for p83, OspA, DbpA and OspC (lane 7). To determine whether OspA-specific antibodies were induced against the three borrelia species (*Bb*ss, *B. garinii* and *B. afzelii*) samples from eight individual horses representative for each single study group (24 horses in total) were analyzed with a

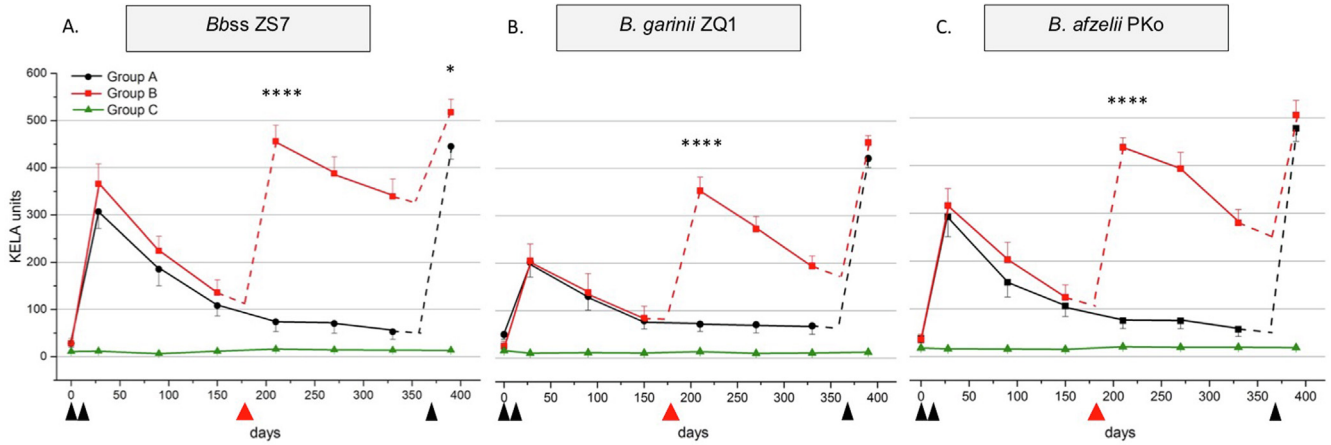

Fig. 2. A–C. OspA specific antibody levels after vaccination with EquiLyme® measured by KELA. The rOspA antigens were derived from either *Bb*ss ZS7 (A) or *B. garinii* ZQ1 (B) or *B. afzelii* PKo (C) and served as detection antigens for IgG antibodies. Horses in group A (regular three-dose-vaccination during one year) are shown with round symbols, horses in group B (modified four-dose-vaccination during one year) are shown with quadrangular symbols and horses in group C (not vaccinated) are shown with triangular symbols. The dash lines indicate the projected antibody kinetics of groups A and B. Arrows indicate to the time points of vaccination. ▲ = additional vaccination of group B. * $p < 0.05$ **** $p < 0.0001$.

species-specific OspA-KELA system. Species-specific OspA antibody levels are shown for *Bbss* in Fig. 2A, for *B. garinii* in Fig. 2B, and for *B. afzelii* in Fig. 2C. Two to five months after the first immunization, OspA antibody levels in Groups A and B dropped off rapidly. Following booster immunization on day 180, horses in Group B developed overall high and long-lasting OspA antibody levels. OspA-specific antibodies in Group B did not drop below pre-vaccination levels for 360 days. After booster vaccination on

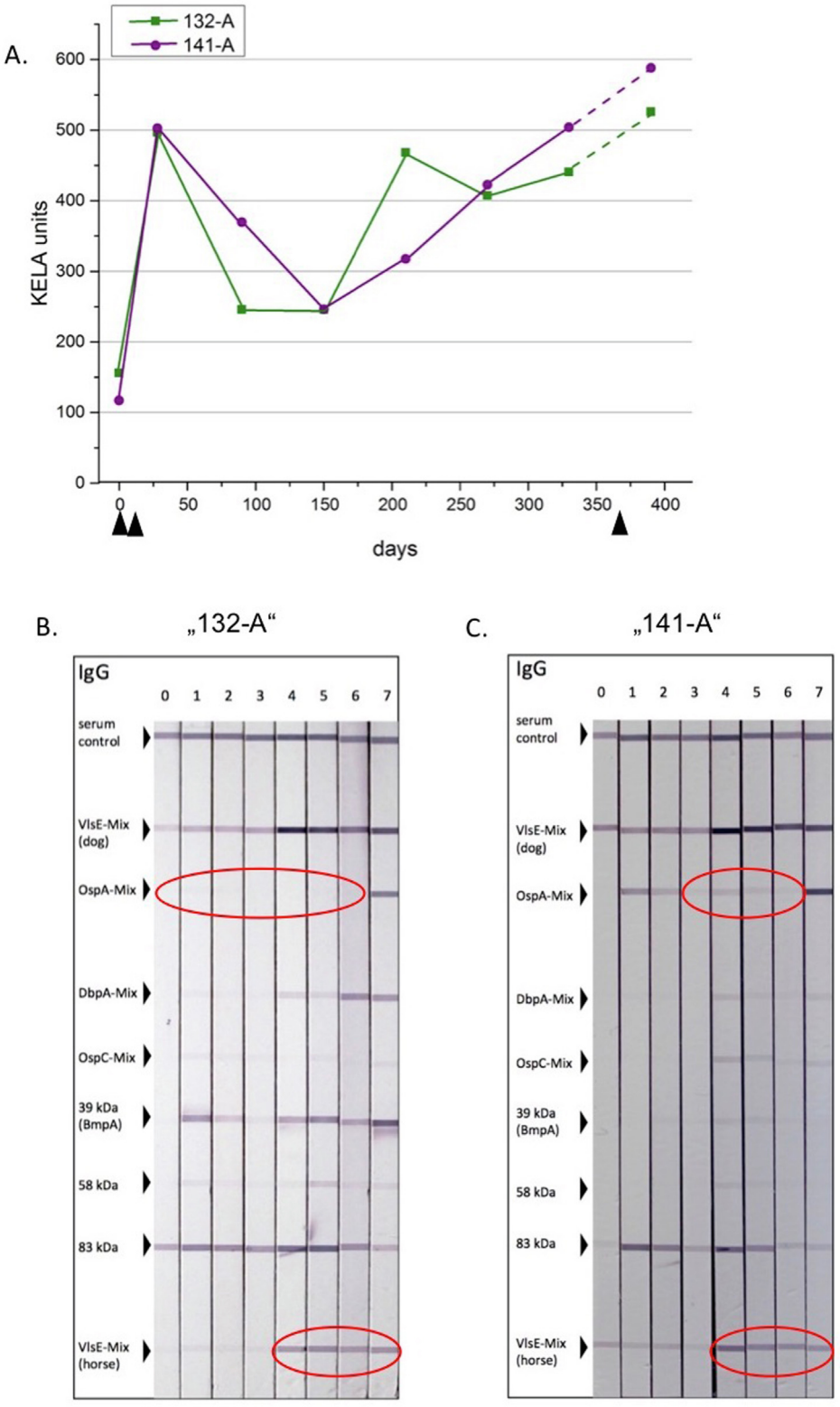

Fig. 3. (A) Antibody levels of serum samples collected from ''132-A" and ''141-A" detected with KELA. IgG antibody detection was accomplished with bacterial lysates produced with *Bbss* N40 enriched with rOspA of *Bbss* (Recombitek® Lyme). Arrows indicate tot he time points of vaccination. The dash lines indicate the projected antibody kinetics. (B,C) Borrelia Veterinary plus OspA LINE of the serum samples collected from horses ''132–A" (B) and ''141-A" (C). The first sample was drawn at day 0 (pre-vaccination); lane1 shows results 28 days after the first vaccination; samples shown in lanes 2–7 (days 90-390) were collected in two-months intervals; samples in lane 7 were collected 28 days after booster vaccination was applied on day 365 +/−3 day.

day 365 ± 3, horses in Group B significantly increased their *Bbss*- and *B. afzelii*-specific OspA antibody levels by 152 KELA units ($p < 0.01$) and 190 KELA units ($p < 0.001$), respectively, compared to measurements to levels detected on day 28 after initial immunization. Interestingly, two serum samples in Group A appeared conspicuous for infection. As shown in Fig. 3A, the two horses developed increasing specific antibody levels starting by day 210 until they reached mean antibody levels of 472 KELA units on day 330, while the other horses in Group A showed a lower mean antibody levels of 332 KELA units. Serum samples collected from horse "132-A" (Fig. 3B) did not reveal an antibody response against OspA after initial vaccination (LIA, lanes 1–3). Starting by day 210, an increasing signal strength for DbpA and VlsE-horse was observed (lanes 4–6). After booster vaccination on day 365, an additional signal for OspA appeared (lane 7). Both horses developed signals for VlsE-horse, DbpA, p39 and p83, a pattern that is indicative for an infection with *Bbsl*. Serum samples collected from horse "141-A" (Fig. 3C) showed clear signals for OspA and p83 and a weak band for VlsE-horse (lane 1) early in the study. By day 210, additional bands were visible for DbpA, OspC, p39 and p58 and signal intensities for VlsE-horse and p83 increased (lane 4). Afterwards signal intensities decreased continuously (lanes 5–6) until booster vaccination led to a strong signal for OspA (lane 7).

4. Discussion

Studies with human volunteers provided evidence that the application of third dose of a LB vaccine within six months after initial immunization resulted in higher and longer-lasting OspA antibody levels compared to the regular two-dose vaccination series [14]. Guarino et al. tested the impact of an additional vaccination in horses by using LB vaccines licensed for dogs. A third dose of each vaccine applied 108 days after initial immunization did not impact on the magnitude of the OspA antibody response compared with two-dose regimen, although longer-lasting OspA antibody levels were observed for one of three canine LB vaccines after a third dose had been applied [15]. In contrast, an additional vaccination with EquiLyme® six months after initial immunization in our study induced high and long-lasting *Bbsl*-specific antibody levels in horses [15].

Studies using mice, dogs and horses have corroborated at large that only sufficiently high antibody levels against OspA provide a protective effect against an infection with *Bbsl* [9,10,16]. As protection against *Bbsl* infection mainly depends on OspA-specific antibodies [17], a special aspect of this study was the measurement of vaccine-induced OspA antibody levels. EquiLyme® consist of whole bacterin lysates derived from cultured *Bbss*, *B. garinii* and *B. afzelii* organisms. As expected, in non-vaccinated horses an OspA-specific antibody response was not initiated. In contrast, immunized horses developed high OspA-specific antibody levels especially against the *Bbss* and *B. afzelii*. The magnitude of the OspA antibody response against *B. garinii* was lower compared to those observed against *Bbss* and *B. afzelii*. Even though EquiLyme® contains approximately equal *Bbss*, *B. garinii* and *B. afzelii* antigen concentrations, it is curious that the OspA-specific antibody response against *B. garinii* was lower when compared to the other two borrelia species. However, *B. garinii* OspA-specific antibody levels were measured using a recombinant OspA antigen obtained from a strain, which was different from that present in the vaccine. Therefore, our non-homologous detection system is probably the reason for the observed low *B. garinii* OspA-specific antibody levels.

During our study, two horses in Group A showed an unexpected increase of their total specific antibodies levels. An increase in intensity and number of LIA signals was observed for a sample collected 210 after the first immunization (July 2017; Fig. 3). Most likely, these horses had contracted an infection with *Bbsl* while they had very low OspA-specific antibody levels and tick exposure was intense at the same time. Thus, it is desirable to revise the regular two-dose-vaccination protocol followed by a booster vaccination twelve months later by introducing an additional booster-vaccination within six months after the first immunization. In this way EquiLyme® can induce high and long-lasting antibodies, which may offer a protective effect against infection with the species *Bbss*, *B. garinii* and *B. afzelii* when an additional immunization is administered.

Contribution of authors

RKS planned the vaccination approach. JK performed the vaccinations, sample collection, sample testing and data analysis. SF attributed to the drafting and editing of the article. All authors contributed to the preparation of the article and approved the final version prior to submission.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.09.087.

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