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Comparative analysis of the immunologic response induced by the Sterne 34F2 live spore *Bacillus anthracis* vaccine in a ruminant model

Okechukwu C. Ndumnego[a,*,1], Susanne M. Köhler[b,1,2], Jannie Crafford[a], Henriette van Heerden[a], Wolfgang Beyer[b]

[a] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, Pretoria, South Africa
[b] Department of Livestock Infectiology and Environmental Hygiene, Institute of Animal Sciences, University of Hohenheim, Stuttgart, Germany

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ABSTRACT

The Sterne 34F2 live spore vaccine (SLSV) developed in 1937 is the most widely used veterinary vaccine against anthrax. However, literature on the immunogenicity of this vaccine in a target ruminant host is scarce. In this study, we evaluated the humoral response to the *Bacillus anthracis* protective antigen (rPA), a recombinant *bacillus* collagen-like protein of anthracis (rBclA), formaldehyde inactivated spores (FIS) prepared from strain 34F2 and a vegetative antigen formulation prepared from a capsule and toxin deficient strain (CDC 1014) in Boer goats. The toxin neutralizing ability of induced antibodies was evaluated using an *in vitro* toxin neutralization assay. The protection afforded by the vaccine was also assessed in vaccinates. Anti-rPA, anti-FIS and lethal toxin neutralizing titres were superior after booster vaccinations, compared to single vaccinations. Qualitative analysis of humoral responses to rPA, rBclA and FIS antigens revealed a preponderance of anti-FIS IgG titres following either single or double vaccinations with the SLSV. Antibodies against FIS and rPA both increased by 350 and 300-fold following revaccinations respectively. There was no response to rBclA following vaccinations with the SLSV. Toxin neutralizing titres increased by 80-fold after single vaccination and 700-fold following a double vaccination. Lethal challenge studies in naïve goats indicated a minimum infective dose of 36 *B. anthracis* spores. Single and double vaccination with the SLSV protected 4/5 and 3/3 of goats challenged with > 800 spores respectively. An early booster vaccination following the first immunization is suggested in order to achieve a robust immunity. Results from this study indicate that this crucial second vaccination can be administered as early as 3 months after the initial vaccination.

1. Introduction

Anthrax is a primary disease of herbivores caused by the Gram-positive bacterium *Bacillus anthracis* (Hambleton et al., 1984, 125–132). The disease develops as a peracute or acute infection in ruminants following an incubation period of 3–5 days (Beyer and Turnbull, 2009, 481–489), which often elapses without clinical signs until shortly before death due to sudden septicemic shock. Clinical signs and course of infection are largely dependent on the species and the route of infection with ambiguous early signs (Hambleton et al., 1984, 125–132). The pathogen expresses two major plasmid encoded virulence factors, a gamma-linked poly-D-glutamic acid capsule [(PGDA) (coded by pX02)] and a tripartite toxin (coded by pX01) comprising of protective antigen (PA), lethal factor (LF) and edema factor (EF) (See review Mourez, 2005, 135–164). Virulence of *B. anthracis* is dependent on the presence of both plasmids (Green et al., 1985, 291–297).

The PDGA is weakly immunogenic and assists in post infection dissemination of *B. anthracis* (Candela and Fouet, 2005, 717–726). The capsule enables the anthrax bacilli to evade immune surveillance mechanisms and enter the circulatory system where it proliferates systemically (Sutherland et al., 2008, 899–906). PA combines with LF to form lethal toxin, a zinc metalloprotease that inactivates most mitogen-activated protein kinase kinases (MAPKK) and the inflammasome-activating NLRP1B leading to impairment and death of susceptible macrophages (Friedlander, 1986, 7123–7126; Chavarría-Smith and Vance, 2013, e1003452). Edema toxin (ET), a calmodulin dependent adenylate cyclase

* Corresponding author.
E-mail address: okeyndumnego@gmail.com (O.C. Ndumnego).
[1] These authors contributed equally to this work.
[2] Current address: Robert Koch Institute, Nordufer 20, 13353, Berlin, Germany.

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formed by the binding of PA to EF, disrupts fluid homeostasis across the host cell membranes (Mourez, 2005, 135–164).

The current anthrax veterinary Sterne live spore vaccine (SLSV) is a non-encapsulated but toxigenic variant 34F2 that was developed in 1937 by Max Sterne (Sterne, 1937, 49–67). This vaccine is still extensively used in the control of anthrax in domestic animals (International Office of Epizootics, 2008, 94–95). Constraints that include limited duration of immunity, failure to induce protective immunity, variation in vaccine quality and adverse reactions in sensitive species, such as llamas (*Lama glama*) and goats (*Capra aegagrus hircus*) have been reported (Cartwright et al., 1987, 715–716, Turnbull, 1991, 533–539). The current OIE manual stipulates annual vaccination of animals against the disease (OIE, 2012, 135–144). Immune parameters and correlates to protection for anthrax in goats are limited in literature because serological methods were not readily available during earlier vaccine trials in domestic species. These trials mainly depended on clinical responses and level of protection after vaccination (Sterne, 1937, 49–67; Sterne et al., 1942, 53).

The principal immune response induced by vaccination of animals with the SLSV is the development of antibodies against PA (International Office of Epizootics, 2008, 94–95). Anti-PA antibodies prevent the development of lethal intoxication and protection against anthrax is mainly provided by the development of immunity to the antigen (Shakya et al., 2007, 5374–5377). The presence of antibodies against the spore (formaldehyde inactivated spores, FIS) and spore-associated antigens such as the *bacillus* collagen-like protein of *anthracis* (BclA) and vegetative antigens has been reported to augment the protection afforded to animals (Brossier et al., 2002, 661–664; Hahn et al., 2006, 4569–4571). Hence we sought to evaluate the humoral immune response in Boer goats directed against these antigens following single or booster vaccinations with the SLSV. Antibody responses were assessed using ELISA. Also, the ability of induced antibodies to neutralize lethal toxin was measured using the *in vitro* toxin neutralization assay (TNA). The level of protection following vaccination was evaluated by challenge with virulent *B. anthracis* spores.

2. Materials and methods

2.1. Animals

Eight-week old female BALB/c mice [(n = 6) (South African Vaccine Producers, Sandringham, South Africa)] were used to confirm the virulence of the *B. anthracis* challenge strain (Welkos et al., 1986, 795–800). Twenty-six 1-year naïve old Boer goats were housed at Onderstepoort Biological Products (OBP), South Africa after screening for anti-rPA83 cross-reactive antibodies. Lethal challenge studies were conducted at a remote site in an endemic area of the Kruger National Park (KNP), South Africa. Animal experiments and clinical score sheets for monitoring experimental animals were drawn up according to the guidelines of the National Research Council of the USA (Clark et al., 1996, 21–34) and approved by the animal use and care committees of the South African national parks, OBP and University of Pretoria (Protocol numbers V041-10 and V065/12) respectively. Approval for Section 20 of the animal disease act 35 of 1984 was granted by the Directorate of Animal Health, Department of Agriculture, Forestry and Fisheries, South Africa (registration number 12/11/1/1/6).

2.2. B. anthracis *vaccine and challenge strains*

Animals were vaccinated using the SLSV as recommended by the manufacturer [(OBP, Onderstepoort, South Africa) (OBP, 2012)]. Challenge was performed with a virulent South African *B. anthracis* strain (20SD) isolated from a sheep in 2001. The presence of both plasmids, pXO1 and pXO2, was confirmed using real time PCR and sequencing (Lekota et al., 2015, 10.1128/genomeA.01313-15).

Spores from the challenge strain were prepared as previously described with minor modifications (Welkos et al., 2011, 4238–4250). Virulence of the spores was confirmed in BALB/c mice and naïve goats. Two groups of 3 mice received an intra-peritoneal challenge of ~500 and ~1000 spores respectively.

Two goats from each of the 3 negative control groups (NCG1-3) were challenged (subcutaneously in the thigh) with 36, 172 and 844 spores respectively. Spore numbers in the respective challenge doses were estimated by counting colony forming units (cfu) prepared from redundant doses. The highest dose of 844 spores was subsequently used for the challenge of the SLSV vaccinated goats (SVG1 to 3, Table 1). Death from anthrax was confirmed after microscopic demonstration of Gram-positive encapsulated rod-shaped bacilli in stained blood smears.

2.3. Experimental design

The immunogenicity and protectiveness of the SLSV were evaluated in four scenarios using 5 goats per group [(SVG1 to 4) (Table 1)]. Two groups were vaccinated once and challenged after 6 (SVG1) and 62 (SVG2) weeks respectively. SVG3 was vaccinated twice at weeks 0 and 58 before lethal challenge 4 weeks later. A fourth group of goats (SVG4) was vaccinated at weeks 0 and 12 to evaluate the titre development in a shortened two vaccination schedule. However, ethical approval for lethal challenge was not obtained for this

Table 1
Vaccination and lethal challenge study design.

Groups	Vaccine	No of animals	Immunization (week)		Serum collection time points for serology (week)															
SVG1	1x SLSV	5	0	–	0	6[c]	8[g]	–	–	–	–	–	–	–	–	–	–	–	–	–
SVG2	1x SLSV	5	0	–	0	4	8	12	16	20	24	28	32	37	48	53	58	62[c]	64[g]	
SVG3	2x SLSV	5[a]	0	58	0	4	8	12	16	20	24	28	32	37	48	53	58	62[c]	64[g]	
SVG4	2x SLSV	5	0	12	0	2	4	8	12	17	20	24[f]								
NCG1	Unvaccinated	3[b]	–	–	0	4	8	12	16	20	24	28	32	37	48	53	58	62[e]	–	
NCG2	Unvaccinated	2	–	–	0	10[d]	–	–	–	–	–	–	–	–	–	–	–	–	–	
NCG3	Unvaccinated	2	–	–	0	11[c]	–	–	–	–	–	–	–	–	–	–	–	–	–	

SLSV-Sterne live spore vaccine. SVG-SLSV vaccinated group. NCG-Negative control group.

[a] Only 3 goats challenged due to incidental deaths from heartwater.
[b] Only 2 goats challenged due to incidental death from heartwater.
[c] Challenge dose 844 spores.
[d] Challenge dose 172 spores.
[e] Challenge dose 36 spores.
[f] Unchallenged.
[g] Sampling time-point of survivors.