Vaccine 36 (2018) 1484–1490

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Strangvac: A recombinant fusion protein vaccine that protects against strangles, caused by *Streptococcus equi*

Carl Robinson [a,*], Lars Frykberg [b], Margareta Flock [c], Bengt Guss [b], Andrew S. Waller [a], Jan-Ingmar Flock [c,d]

[a] Department of Bacteriology, Animal Health Trust, Lanwades Park, Kentford, Newmarket CB8 7UU, UK
[b] Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, P.O. Box 7036, SE-750 07 Uppsala, Sweden
[c] Department of Microbiology, Tumor and Cellbiology, Karolinska Institutet, P.O. Box 280, SE-171 77 Stockholm, Sweden
[d] Intervacc AB, PO Box 112, SE-129 22 Hägersten, Sweden

ARTICLE INFO

Article history:
Received 27 October 2017
Received in revised form 20 December 2017
Accepted 10 January 2018
Available online 2 February 2018

Keywords:
Strangles
Streptococcus equi
Recombinant
Vaccine

ABSTRACT

The host-restricted pathogen *Streptococcus equi* causes strangles in the horse, which is characterised by abscessation of the lymph nodes of the head and neck. The disease is endemic throughout the world causing considerable welfare and economic cost to the horse industry. Here we report the results of three studies where ponies were vaccinated with combinations of recombinant fusion proteins to optimise vaccine production and the level of protection conferred. Optimal protection was conferred by a prototype multicomponent subunit vaccine, Strangvac 4, which contained eight proteins CNE, SclC, SclF, SclI, EAG (fused as CCE), SEQ_402, SEQ_0256 (fused as Eq85) and IdeE. Across the three experiments only three of 16 ponies vaccinated with Strangvac 4 became pyretic compared to all 16 placebo-vaccinated control ponies ($P < .001$). *S. equi* was recovered from the lymph nodes of eight Strangvac 4-vaccinated and 15 control ponies ($P = .016$). None of the ponies vaccinated with Strangvac 4, or the other prototype vaccines developed adverse reactions following vaccination. Our data provide evidence in support of the further clinical development of the Strangvac 4 vaccine.

1. Introduction

Streptococcus equi subsp *equi* (*S. equi*) is the causative agent of strangles, which is one of the most frequently identified infectious diseases of the horse [1]. *S. equi* enters a naïve animal via the nose or mouth and attaches to the tonsillar epithelial cells spreading through the local lymphatic system [2]. Infection of the lymph nodes in the head and neck leads to the formation of abscesses that can restrict the airway. Abscesses burst, releasing a purulent discharge that contributes to the highly contagious nature of the disease [1,3]. Consequently, strangles causes significant suffering of the horse and substantial economic cost to the UK equine industry which is worth over £7 billion annually to the UK economy alone.

S. equi is believed to have evolved from an ancestral strain of *S. equi* subsp. *zooepidemicus* via a process of gene gain and loss [4]. Although strangles is an ancient disease [5,6], Bayesian statistical analysis of the genomes of a diverse collection of contemporary strains of *S. equi*, including isolates from UK, Australia, Belgium, Canada, Ireland, New Zealand, Saudi Arabia, Sweden, and the United States, shows that they share a common ancestor that dates to the late 19th or early 20th Century [7]. Therefore, the modern population of *S. equi* has relatively little genome diversity [7], suggesting that strangles vaccines would likely confer cross-protection against all of the circulating strains.

Following recovery from infection, horses typically have immunity against re-infection [8]. This knowledge formed the basis for the development of live attenuated vaccines that protect against strangles. The Pinnacle IN vaccine (Zoetis) is available for use in horses in the USA and New Zealand and Equilis StrepE (MSD Animal Health) is available to protect horses against strangles in the European Union [9]. Both vaccines confer protection, but must be given via the intranasal and submucosal routes, respectively to minimise the risk of complications. Adverse reactions following vaccination with Equlis StrepE have been documented [7,10,11] and the Pinnacle IN vaccine strain has been associated with clinical Strangles [12] and was recovered from 61% of strangles cases in vaccinated horses in one study [13]. Furthermore, the immune responses of vaccinated animals cannot be differentiated from those that have been infected with wild-type strains, confounding the identification of vaccinated animals that have been exposed to *S. equi*, so called DIVA capability (Differentiation of Infected from Vaccinated Animals) [14]. Therefore, the development of a vaccine

* Corresponding author.
E-mail address: carl.robinson@aht.org.uk (C. Robinson).

https://doi.org/10.1016/j.vaccine.2018.01.030
0264-410X/

that can be administered via the intramuscular route, protects against infection and does not interfere with diagnostic tests remains an important unmet goal.

A prototype vaccine, Septavac, which was composed of seven recombinant proteins derived from *S. equi*, conferred significant levels of protection in Welsh mountain ponies [15]. However, the commercial costs of manufacturing a vaccine composed of seven proteins would be prohibitively high. Here we report the protection conferred by vaccination with combinations of five fusion proteins comprising eleven different *S. equi* antigens in Welsh mountain ponies.

2. Results

2.1. Vaccination with Strangvac fusion proteins confers protection against the clinical signs of strangles

Nine of the eleven recombinant *S. equi* antigens studied were fused together in five different combinations (Table 1 and Supplementary Table S1). Additionally, IdeE, IdeE2 and EndoS were included as unfused components in Strangvac 2 and 4, Strangvac 2, and Strangvac 5 and 8 respectively, as shown in Table 1. Ponies were grouped into three experiments and vaccinated on two (Experiment III) or three (Experiments I and II) occasions via subcutaneous injection and intranasal spray with combinations of the Strangvac proteins or an adjuvant-only placebo as illustrated in Table 1 and Fig. 1.

Ponies were challenged two weeks post-final vaccination by the administration of 4 ml of Todd Hewitt broth containing 10% foetal calf serum and 1×10^8 cfu of *S. equi* strain 4047 (*Se*4047), which was split and 2 ml sprayed into the left and right nostrils. Ponies were monitored twice daily for the onset of clinical signs of disease and rectal temperatures were recorded daily and utilised as the primary indicator of infection with *Se*4047. Comparisons between Strangvac-vaccinated and placebo-vaccinated ponies were made on days 8 (the day on which the last placebo-vaccinated ponies remained alive for Experiment I and blood samples were taken for analysis) and 20 (at the end of Experiment 1 and the final day that a blood sample was taken) for all Experiments for consistency.

The normal temperature of a healthy horse is approximately 37.5 °C to 38.5 °C. All placebo-vaccinated controls in Experiments I, II and III (n = 6, 5 and 5, respectively) became pyretic (Fig. 2), defined as a temperature exceeding 38.9 °C, during the course of each experiment. Strangvac 2-vaccinated and Strangvac 5-vaccinated ponies in Experiments 1 and 2, respectively had significantly less pyrexia than placebo-vaccinated ponies on both days 8 and 20 (all $P < .03$, Supplementary Table S2). Strangvac 8-vaccinated ponies had significantly less pyrexia than placebo-vaccinated ponies on day 8 ($P = .047$), but not day 20 in Experiment II. Strangvac 7-vaccinated ponies had no significant reduction in pyrexia on either day 8 or 20 in Experiment II. Strangvac 4-vaccinated ponies had significantly less pyrexia than placebo-vaccinated ponies on both day 8 and 20 in each of the Experiments (all $P \leq .02$). Combining the data across the three experiments for Strangvac 4 revealed a highly significant reduction in elevated temperatures ($P < .001$) on both days 8 and 20. Significant differences in the incidence of pyrexia were identified in Experiments I ($P = .015$) and II ($P = .008$), but not Experiment III (Supplementary Table S3). Combining the data across the three experiments revealed a highly significant reduction in the overall incidence of

Table 1
Composition of the different vaccine formulations. # – indicates components fused together.

Vaccine	Experiment	Fusion proteins in the vaccine	*S. equi* proteins contained within the vaccine#	No of ponies vaccinated
Strangvac 2	I	Eq85, CCE, IdeE, IdeE2	Eq8-Eq5, CNE-SclC-SclF-SclI-EAG, IdeE, IdeE2	6
Strangvac 4	I	Eq85, CCE, IdeE	Eq8-Eq5 CNE-SclC-SclF-SclI-EAG, IdeE	6
Placebo	I	None	None	6
Strangvac 4	II	Eq85, CCE, IdeE	Eq8-Eq5 CNE-SclC-SclF-SclI-EAG, IdeE	5
Strangvac 5	II	CneEag, IE5, EndoSe	CNE-EAG, Eq5-IdeE, EndoSe	6
Strangvac 7	II	CPCE, IE5	CNE-Eq54-SclC-SclI-EAG, IdeE-Eq5	6
Strangvac 8	II	CPCE, IE5, EndoSe	IdeE-Eq5 CNE-Eq54-SclC-SclI-EAG, EndoSe	6
Placebo	II	None	None	5
Strangvac 4	III	Eq85, CCE, IdeE	Eq8-Eq5 CNE-SclC-SclF-SclI-EAG, IdeE	5
Placebo	III	None	None	5

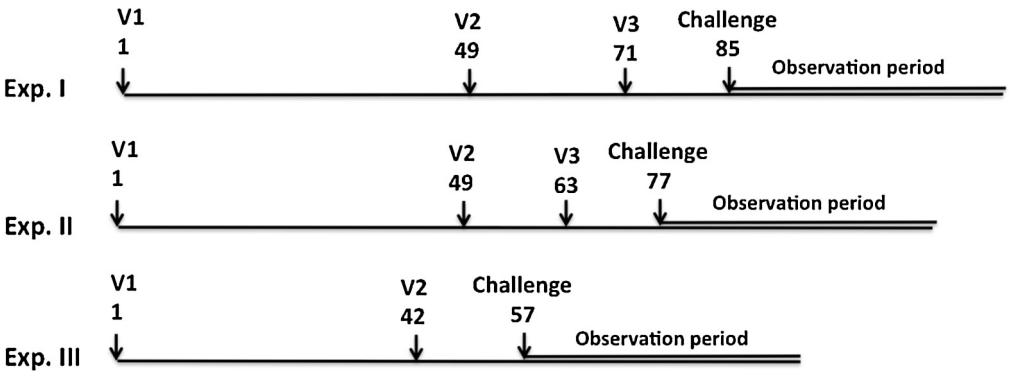

Fig. 1. Immunization schedule. Ponies were vaccinated as indicated, followed by experimental infection 14 days after the last vaccination. Ponies were observed for up to 28 days or until the time of euthanasia. V1, V2 and V3 indicate the time of first, second and third vaccinations.