PRELIMINARY ANALYSIS OF THE DURATION OF PROTECTION OF VAXSAFE® ST VACCINE AGAINST SALMONELLA SHEDDING IN LAYERS.

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Summary

Vaxsafe® ST (Bioproperties Pty Ltd) is an *aro*A deletion mutant vaccine for the control of *Salmonella* Typhimurium in chickens. It is registered for spray and drinking water applications and has been shown to aid in the control of Salmonella in short lived birds (such as broilers). The main objective of this study was to test efficacy of Vaxsafe® ST with a new vaccination program aimed at lengthening the duration of immunity for the full productive life of layers. The first dose was delivered by spray on chicks at one day of age followed by two doses administered orally at 2 and 6 weeks of age (woa) respectively, and a fourth dose administered by intramuscular (IM) injection at 10 woa. All vaccination doses were administered at 10^7 cfu/dose. For the fourth IM dose, the vaccine was delivered in two different formats, mixed in diluent or in a mixture with Nobilis® EDS+ND vaccine (Intervet). At 16, 30, 45 and 65 woa respectively (that is at 6, 20, 35 and 55 weeks post IM vaccination) a sample of the vaccinated birds, was transported to a research facility and challenged with wild type *S.* Typhimurium (ST) or *S.* Infantis (SI). Salmonella shedding of vaccinated birds were compared with a control unvaccinated (challenged) group in order to assess efficacy of the vaccine. Vaxsafe® ST delivered in either diluent or with Nobilis® EDS+ND killed vaccine, provided protection against strong homologous (ST) and heterologous (SI) challenges compared to the unvaccinated birds.

I. INTRODUCTION

Vaxsafe® ST is a registered vaccine produced by Bioproperties Pty Ltd. The statement of claims for this vaccine is as "An aid in the control of colonisation by *S.* Typhimurium. The vaccine has been shown to reduce the excretion of virulent *S.* Typhimurium and provide chickens with an aid in protection against challenge by this strain". The registered method of administration is by spray on chickens at one day of age followed by an oral administration at 2 woa. This regimen was designed to protect short-lived birds (broilers) against *S.* Typhimurium challenge, but to date has not been widely adopted. Recently it has been used by injection in broiler breeder salmonella control programmes in Australia, combined with killed vaccines with great success. In order to extend the duration of immunity till the end of life for layers, a new vaccination program needs to be developed and tested.

Previous studies have shown that Vaxsafe® ST when given with regimens including an intra-muscular injection can provide useful protection against *S.* Typhimurium challenge and heterologous challenge with *S.* Infantis, and to a lesser degree *S.* Virchow challenge (Sharp *et al*. 2012). These experiments did not define the duration of this immunity and the cost of administration would be an impediment for general adoption in the layer industries.

In this study, the current vaccination program for Vaxsafe® ST was modified to include a third dose delivered by drinking water at 6 woa followed by a fourth dose administered by intramuscular (IM) injection at 10 woa. For the first three vaccinations, Vaxsafe® ST was delivered at a dose of 10^7 cfu in water. For the fourth vaccination, it was tested at a dose of 10^7 delivered in diluent, or mixed with Nobilis® EDS+ND killed vaccine. Nobilis® EDS+ND is a combined vaccine for the immunisation of chickens against Newcastle Disease and Egg Drop Syndrome '76. The Nobilis® EDS+ND killed vaccine consists of an inactivated antigens prepared as a water in oil emulsion.

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II. METHODS

A total of 26,000 Salmonella free commercial Hy-Line layer birds were vaccinated at the hatchery by coarse spray at day of age. The birds were transported to a commercial farm and reared in cages in shed A as per the normal farm management procedures. A group of 26,000 birds were left unvaccinated and were transported separately to the same farm and reared in cages in shed B. At 2 and 6 weeks of age all birds in shed A were vaccinated with Vaxsafe® ST (10^7 cfu/dose) in drinking water as per manufactures instructions. At 10 weeks of age, two groups of 125 birds were selected from shed A and vaccinated by IM injection (0.5mL/dose/bird) into the pectoral muscle with one of two Vaxsafe® ST formulations (in Marek's diluent or mixed with Nobilis® ND/EDS killed vaccine). A vial of freeze dried Vaxsafe® ST vaccine (1000 doses/vial) was resuspended in 3 mL of Marek's diluent and then added into 1000 doses of the killed vaccine and mixed by shaking.

Birds in shed B remained unvaccinated against Salmonella. At each of 6, 20, 35 and 55 weeks post IM vaccination, ten birds from each of the vaccinated groups and ten birds from shed B (control shed) were transported to a research facility, and placed into isolators. At 48 and 24 hours before the administration of the challenge, all chickens from all groups received vancomycin (60 mg per bird *per os* in 0.6mL). (Modified from Marcq *et al*., 2011). The challenged consisted of freshly cultured 10^9 cfu/dose of wild-type ST or SI. Chickens were monitored for clinical signs throughout the study and screened for Salmonella shedding with cloacal swabs being taken on days 0, 2, 7 and 14 post challenges. The presence and concentration of live Salmonella in swabs was determined by titration in Rappaport-Vassiliadis (RV) media (after resuscitation in Peptone broth) and identity confirmation on XLD and SMID plates followed by specific wild-type ST or SI PCR based methods. All data collected from the vaccinated and challenged birds were compared with those collected from the control groups in order to assess efficacy of the vaccine.

III. RESULTS

No clinical signs were observed in any of the vaccinated groups immediately after vaccination, or throughout the study. Prior to each challenge time-point, cloacal swabs were collected from chickens on the farm from both sheds A and B and screened to ensure that the birds were not infected with Salmonella. Also just prior to challenge, cloacal swabs were collected from birds in all groups. All these results were negative for Salmonella. In the positive control groups, the infection profile for ST was quite different to that of SI in terms of the number of organisms shedding, as well as the duration of the infection. Nonetheless, shedding from the positive control ST and SI groups was relatively high, indicating a good rate of challenge.

a) Shedding of *S.* Typhimurium and *S.* Infantis

There was a reduction in mean shedding of ST (see Figure 1. Panel a) in vaccinated chickens at most time points. Chickens challenged at 6 weeks after the IM vaccination with Vaxsafe® ST combined with Nobilis® EDS+ND showed a significant reduction the amount of ST shedding by day 14 after challenge. However, in chickens challenged at 20, 35 and 55 weeks after the IM vaccination, Vaxsafe® ST delivered in either diluent or combined with Nobilis® EDS+ND showed reduction in ST shedding by day 14 after challenge.

There was a reduction in shedding of SI in all vaccinated groups over the 3 sampling time points (days 2, 7 and 14) when compared with the unvaccinated positive control group. Shedding from the positive control remained high even at 14 days post challenge (see Figure 1. Panel b). Significant reduction ($p<0.05$) in SI shedding in vaccinated groups compared with the unvaccinated groups (positive control) was obtained when Vaxsafe® ST was delivered in diluent for challenges at 6 and 20 weeks after vaccination. Interestingly, a significant reduction ($p<0.05$)

in SI shedding compared with the positive control groups was obtained for both injected presentations of Vaxsafe® ST (in diluent and mixed with Nobilis® EDS+ND) for the challenge at 35 and 55 weeks after vaccination.

IV. DISCUSSION

This experiment lacked the power to cope with the stochastic nature of the Salmonella challenge shedding data, but trends were consistent and the demonstration of significant effects at later time points (in the case of the heterologous SI challenge) would allow the reasonable conclusion that some protection existed at earlier points. This problem may be overcome by Bayesian analysis of the data similar to a recent paper by Arnold *et al* (2014). These trends were a decrease in excretion in day 2 swabs in ST challenge in vaccinated groups and then all groups had similar rates of clearance. The SI challenge organism appears to be able to colonize the hens with cloacal shedding results from day 7 and day 14 being similar while in vaccinated groups these usually appeared to be being cleared. This suggests that the immunity from the Vaxsafe® ST vaccine primes the hen and this broad immunity can be rapidly mobilised in the gut of the chicken to reduce excretion of heterologous serovars. By day 14 the rates of shedding were similar in most cases whether the vaccine had been mixed with the killed NDV/EDS or just administered separately in diluent (Figure 1).

The vaccination regimen described illustrates potential for protection against ST egg contamination, and may provide a broad general Salmonella protection. Large numbers of layer hens in the field have now been vaccinated with similar IM regimens and it is well tolerated and rearing is reported as unaffected. Transient depression after vaccination (Groves and Sharp 2012) has not been observed in this experiment and has not a problem in the field to date. The Egg Layer industry should have the confidence from this work, previous work (Sharpe *et al* 2012), and positive experiences with similar vaccination regimens using Vaxsafe® ST in the broiler breeder industry, to begin investigating the advantages of this technology.

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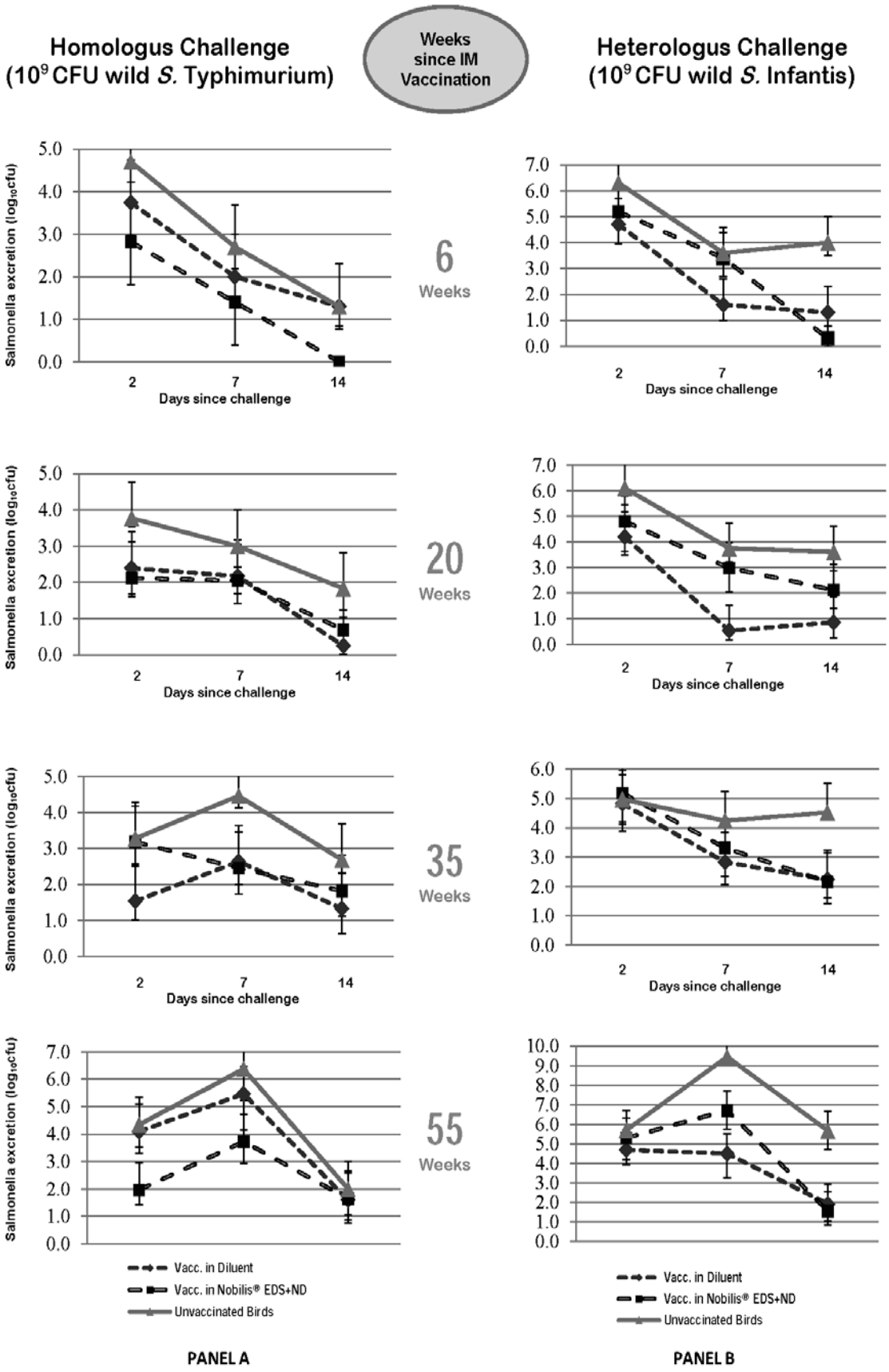

Figure 1 - Shedding of ST and SI in vaccinated and control groups challenged with wild type ST or SI at 6, 20, 35 and 55 weeks after IM vaccination with Vaxsafe® ST delivered either in diluent or mixed with Nobilis® EDS+ND killed vaccine. Means with error bars (standard error). Sampling/Data points are at 2, 7 and 14 days after challenge.