CAMBRIDGE TECHNOLOGIES
PRECISION VACCINOLOGY

Research Report

Novel Baculovirus Vectored Wart Vaccine

INTRODUCTION

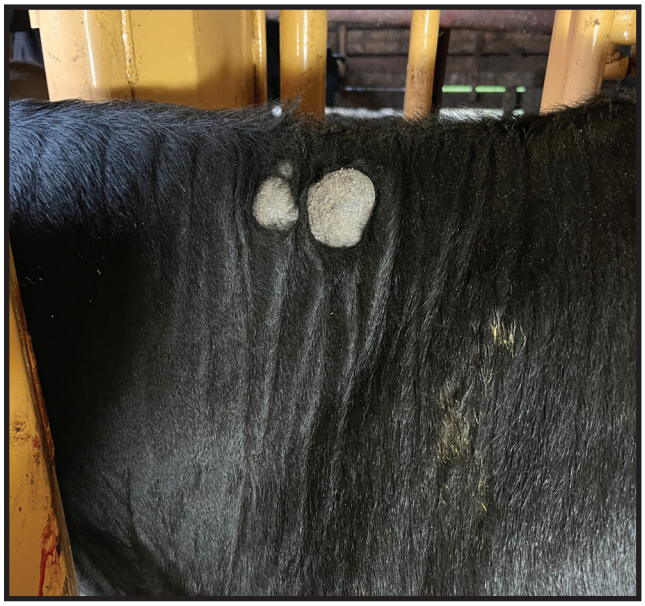
REPORT: Reasonable expectation of efficacy demonstrated by proposed Killed Baculovirus Vector vaccine for viral papillomas (warts)

The need for a modernized approach to prevention of bovine papillomas is well-recognized among cattlemen and veterinarians alike. Historically, wart-extract vaccines have been inconsistent in reliability, supply, and in relation to risks of transmission of adventitious agents. Cambridge Technologies has completed a pair of studies that show a reasonable expectation of efficacy (REE) for a Wart Vaccine, Killed Baculovirus Vector Vaccine, Product Code 1985.R0. The first study used a pseudovirus serum neutralization (pSN) assay to demonstrate the production of pSN neutralizing antibodies of >1:640 in vaccinated calves while unvaccinated placebo calves remained seronegative. A supplemental challenge study confirmed REE when used to vaccinate calves three months of age or older, as an aid in the control of viral papillomas (warts).

This research has been submitted to the USDA CVB as part of the process for receiving a conditional license for the vaccine.

STUDY ONE

The first study evaluated the generation of neutralizing antibody titers. Sixteen angus-cross, healthy colostrum-deprived calves were enrolled and housed at Cambridge Technologies' Research Farm. The calves had not received any vaccines prior to arrival and were confirmed deltapapillomavirus 4 (DPV4) naïve. Both male and female calves were used. Eleven calves were randomly assigned to the test group, and the remaining five were designated as controls and served to ensure no environmental exposure to bovine papillomavirus occurred during the study.



Bovine papillomas can lead to devaluation of hide quality, have a large negative impact on show animals, and can interfere with the ability of the animal to secure a health certificate for transport.

The eleven calves in the test group received an experimental vaccine produced at the R&D Facility of Cambridge Technologies and represents the product that will be prepared in the licensed facility for the conditionally licensed product. Antigen was produced from the master seed virus at the maximum allowable passage (msv+4). Antigen titer was determined by titration in SF9 cells and results read by IFA using baculovirus GP64 conjugate (Santa Cruz biotech).

The five calves in the control group received a placebo where SFM II 900 media was used to replace antigen in the vaccine formulation.

All calves were comingled in a single pen in a barn for the duration of the study. This was a vaccination-only study that consisted of calves receiving 1 x 4mL dose of vaccine (DPV4 or placebo) subcutaneously in the neck (alternating sides for each dose) every two weeks for a total of three administrations. Both the experimental vaccine and placebo were tested for purity and safety.

Animal care staff observed the calves, their husbandry, and the general conditions existent within all test facilities daily and observations were recorded. Observations were done around the same time of day every day, and any abnormalities were brought to the attention of the study's principal investigator. Animals were also observed on day of vaccination and three days thereafter for any adverse reactions.

All calves had a serum sample taken upon arrival, at time of first vaccination (day 0), day of third vaccination (day 28), and two weeks post final vaccination (day 42). All serum was tested for antibodies to DPV4 with the pSN assay by Cambridge Technologies staff.

The schedule was as follows:

Study Date	Activity
-25 to -22	Prior to shipping, calves were ear-tagged
-21 to -1	Calves arrived at the study site to acclimate prior to study onset. Health upon receipt was recorded and calves were bled for screening with pSN and determined to be negative for neutralizing antibodies to L1 and L2. All calves were comingled in a single pen in a barn. All animals were enrolled in the study and treatment group assigned by the randomization of the study statistician.
0	Observed and recorded general observations; obtained a serum sample from each animal, administered 4mL of masked treatment SQ in the right side of the neck (DPV4 vaccine or placebo, per the randomization table) to all enrolled calves. Noted some coughing. Observed for any injection reactions.
1	Study veterinarian obtained a deep nasal swab from three calves for testing and treated all calves with ResFlor Gold.
1-3	Observed and recorded any post-vaccination reactions and general health observations.
4-13	Observed and recorded any general health observations.and recorded any post-vaccination reactions and general health observations.
14	Administered 4mL masked treatment SQ in left side of the neck (either DPV4 vaccine or placebo, per randomization table) to all calves. Observed for any injection reactions.
15-17	Observed and recorded any post-vaccination reactions and general health observations.
18-27	Observed and recorded any general health observations.
28	Observed and recorded general observations; obtained a serum sample from each animal, administered 4mL of masked treatment SQ in right side of the neck (DPV4 or placebo, per randomization table) to all enrolled calves. Observed for any injection reactions.
29-31	Observed and recorded any post-vaccination reactions and general health observations.
32-41	Observed and recorded any general health observations.
42	Observed and recorded general health observations. Bled all calves.

All calves were either 102 or 103 days of age at the onset of the study, and were 14, 16, and 18 weeks of age at each respective vaccination. Only the animal treatment technician knew which vaccine was administered to each animal and provided each treatment to the animal worker to administer to each animal, based off of the randomization. All other study personnel were blinded to the treatment given to each animal.

The pSN assay was performed on all collected serum. In this study, 11/11 DPV4-vaccinated calves seroconverted (pSN titer >=80) after two doses (day 28) of the test vaccine. The titers continued to increase following administration of the third dose. All placebo-vaccinated calves remained negative to DPV4 by the pSN assay.

Table 1. pSN titers from first REE study

Treatment Group	Study Day	N	25th Percentile	Median	75th Percentile	Minimum	Maximum
T1 REE VX	-21	11	<=20	<=20	<=20	<=20	<=20
	0	11	<=20	<=20	<=20	<=20	<=20
	28	11	160	640	640	80	640
	42	11	1280	1280	2560	640	2560
T2 Placebo	-21	5	<=20	<=20	<=20	<=20	<=20
	0	5	<=20	<=20	<=20	<=20	<=20
	28	5	<=20	<=20	<=20	<=20	<=20
	42	5	<=20	<=20	<=20	<=20	<=20

This study served to establish a reasonable expectation of efficacy for a baculovirus-expressed DPV4 L1 vaccine, Product Code 1985.R0, by demonstrating its production of pSN neutralizing antibodies of >1:640 in all vaccinated calves while all unvaccinated placebo calves remained seronegative. No papillomas were detected at any time on any enrolled animal. Additionally, no systemic adverse reactions to vaccine administration nor any injection site reactions were noted in any calves.

STUDY TWO

Following the conclusion of Study #1, a subsequent study involving the same 16 calves served to both establish a challenge model for DPV4 in bovines, and to demonstrate that the seroconversion observed in the first study was adequate to protect those calves from a prototypic papilloma-inducing challenge.

One-hundred and six days subsequent to the final treatment administered during the first REE study, each calf was intradermally challenged with each of two prototypic challenge preparations: one preparation was a wart homogenate from a 2022 field case, and the other was bovine papillomas material obtained from ATCC (strain 324). Calves were observed and challenge sites palpated multiple times throughout the duration of the study. On the final day, all calves were bled for pSN testing, and samples of the papilloma were taken for sequencing from any calf showing a palpable abnormality at the challenge site.

The presence of papillomas was first observed on day 38 post-challenge.

The 16 calves that had been enrolled in the first study were moved to a challenge facility for the duration of this second study. All animals were co-mingled together in a single pen with a lean-to style barn, and they were approximately 7.5 months of age when challenge was administered.

To administer the challenge, a small section of skin was shaved at the base of each calf's neck on the left side. Four separate patches of skin, approximately 1cm2 each, in the shaved area were abraded by scratching with a 20-gauge needle. The top two abraded spots on each animal had approximately 0.5mL of ATCC-challenge material dropped and scraped into the abrasion with a syringe fitted with a 20-gauge needle. The bottom two abraded spots on each animal had approximately 0.5mL of papilloma tissue homogenate-challenge material dropped and scraped into the abrasion with a syringe with a 20-gauge needle.

As with the first study, animal care staff performed daily observations of the calves, their husbandry, and the general conditions existent within all test facilities. All challenge sites were palpated on day 45 post-challenge by the principal investigator, and again by a veterinarian on day 60. Again, all observers, laboratory staff, and veterinarian were blinded to group assignments.

The schedule was as follows:

Study Date	Activity
1-38	All calves were challenged with two challenge preparations.
39-44	Animals were observed daily for general health observations, but animals were not individually examined. On day 38, it was noted by a caretaker that three animals appeared to have papillomas or growths in the challenge area.
45	Animals were observed daily for general health observations, but not individually examined.
58	All animals were processed through a chute, and each was examined by the principal investigator to confirm presence or absence of papillomas in the challenge area.
60	Two animals, which were being utilized in a new study and beginning a treatment regime for this new study, were processed through a chute, and each challenge site was examined and palpated by a veterinarian kept fully blinded to prior treatment group and blind to calves' serostatus. Photos of ear tag and challenge area were taken, and blood samples were also collected from each calf.
4-13	Remaining 14 animals were processed through a chute and each challenge site was examined and palpated by a veterinarian kept fully blinded to prior treatment group and blind to calves' serostatus. Photos of ear tag and challenge area were taken for each calf, and samples from any challenge site abnormalities were excised for sequencing. Blood samples were collected from each calf.

The presence of warts was first observed by the daily caretaker on day 38 post-challenge, on three animals. By day 45, five animals were found to be positive for the presence of papillomas. It was difficult to discern whether the palpable abnormalities were located in the upper or lower challenge administration site.

At day 60, these five animals were still the only ones to have developed warts. Of those, three of the five developed four total warts, one in each of the four challenge sites. The other two calves developed warts from one of the challenge strains (two warts in the two challenge sites for that strain). All five animals were in the original placebo-vaccinated group.

All of the excised tissue samples from the palpably abnormal challenge sites yielded sequencing results consistent with the presence of papillomavirus in the sample, leading to a diagnosis of challenge-induced papilloma.

Table 2. Calf pSN titers after the third vaccination pre-challenge (study 1) and the number of papillomas and sequence type of papillomas detected post-challenge

Calf ID	Group	pSN post 3rd VX (92 days pre-challenge)	papilloma + sites (final palpation by blinded observer)	DPV sequence detected
352	Vaccinate	1280	0	NA
354	Vaccinate	1280	0	NA
355	Vaccinate	2560	0	NA
356	Control	≤20	2	BPV1+
357	Vaccinate	640	0	NA
358	Control	≤20	4	BPV1+ BPV2+
359	Control	≤20	4	BPV1+ BPV2+
360	Vaccinate	1280	0	NA
361	Control	≤20	2	BPV2+
362	Vaccinate	2560	0	NA
363	Vaccinate	1280	0	NA
364	Vaccinate	1280	0	NA
365	Control	≤20	4	BPV1+ BPV2+
366	Vaccinate	2560	0	NA
367	Vaccinate	1280	0	NA
368	Vaccinate	2560	0	NA

The DPV4 sequences obtained from warts derived from ATCC challenge were >99% identical to the BPV1 sequence of ATCC strain for calves 356, 358, 359, and 365. The DPV4 sequences obtained from warts resulting from the homogenate-challenge were 99% identical to the BPV2 homogenate challenge for calves 358, 359, and 361.

The pSN was not performed on the 60-day post-challenge blood samples, as papillomavirus is generally sequestered from the immune system in the epidermis. The serum was stored at -80 degrees Celsius, should further testing be deemed warranted.

CONCLUSION

These two studies, when considered in conjunction with each other, confirm there is a reasonable expectation of efficacy for Code 1985.R0, when used to vaccinate calves three months of age or older, as an aid in the control of viral papillomas (warts).