Analysis of Recombinant Canarypox Vectored West Nile Virus Vaccine Stability Post-Reconstitution

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ABSTRACT

Veterinarians in mobile equine practices are interested in data regarding the stability of modified live vaccines after reconstitution under field conditions. Therefore, the stability of a reconstituted recombinant canarypox vectored West Nile virus vaccine (RECOMBITEK® Equine WNV vaccine) was assessed under 5 separate experimental conditions. Each study entailed exposing 1 to 4 vials of reconstituted vaccine to experimentally controlled environmental conditions, simulating storage conditions that are inconsistent with the label recommendation but that could be encountered in the field. For the purposes of these studies, the conditions examined were exposure over time to: sunlight, room temperature (20°C [68°F]), elevated temperature (37°C [98°F]), refrigeration (2°C to 7°C [36°F to 45°F]), and a freeze-thaw cycle. Titer analyses were performed on aliquots drawn from reconstituted vials of commercially available vaccine. These studies were performed to provide useful data for equine veterinarians, and are presented here as observations and not intended to replace label instructions or estimating the result of the impact of these environmental issues in the course of their daily practice. The results of the UV exposure study demonstrate and reinforce the need to protect reconstituted RECOMBITEK® Equine WNV vaccine from direct sunlight. However, if not concurrently exposed to UV light, it is reasonable to expect reconstituted recombinant canarypox vectored West Nile virus vaccine to remain stable for up to 8 hours at room temperature, up to 30 hours when refrigerated, up to 6 hours at elevated environmental temperatures of 37°C (98°F), at thawing, and up to 1 hour after thawing, in the event the vaccine should be frozen.

INTRODUCTION

RECOMBITEK® Equine WNV vaccine consists of a recombinant modified-live

canarypox virus expressing preM and E genes derived from a 1999 New York isolate of West Nile virus (WNV).[1] The vaccine has been demonstrated to provide rapid onset of protection and duration of immunity for a year after a 2-dose vaccination protocol. The vaccine also was demonstrated to protect horses from WNV-viremia when horses were challenged with WNV by direct infection from mosquitoes 26 days after a single vaccine administration.[2] These studies were conducted using vaccines prepared at the minimum protective dose (MPD), which, according to federal regulations, is the lowest vaccine titer of a live vaccine that provides protection. RECOMBITEK® Equine WNV vaccine is presented in a lyophilized cake that is reconstituted with sterile diluent immediately prior to use.

It is important that vaccines always be handled properly to ensure quality, efficacy, and safety. Vaccines should always be stored and maintained under refrigerated conditions to ensure the best possible stability of the components, whether the vaccine contains killed or live virus. Ideally, all lyophilized vaccines should be reconstituted immediately prior to administration as per label recommendations. In field equine practice, numerous situations can occur causing the administration of vaccines to be delayed after the vaccine has been reconstituted and exposing reconstituted vaccines to unforeseen temperature conditions and sunlight.

In response to frequently asked questions, several studies were performed to determine the stability of reconstituted recombinant canarypox vectored West Nile virus vaccine (RECOMBITEK® Equine WNV vaccine). Five studies were conducted, using similar assessment methods, to explore the potential effects of various environmental conditions a practitioner might face that would impact the stability of reconstituted RECOMBITEK® Equine WNV vaccine.

MATERIALS AND METHODS

Commercially available RECOMBITEK® Equine WNV vaccine was used in each of these studies. The amount of live canarypox virus in the vaccine (vial titer) was evaluated following reconstitution under the following environmental conditions: exposure to direct sunlight (UV radiation) up to 6 hours; room temperature (20°C [68°F]), up to 8 hours; elevated temperature (37°C [98°F]) up to 6 hours; refrigeration (2° to 7°C [38° to 45°F]) up to 30 hours; and freeze/thaw cycle (frozen at -70°C [-94°F] for 2 hours then thawed at room temperature and held for 1 hour).

Definitions of Testing Conditions

UV Radiation (Study 1). Testing was performed with clear glass vaccine vials on July 15, 2004. Samples were reconstituted and kept outside on a clear day, with no intermittent cloud cover. Temperature was modulated to reduce temperature effect on the vial by placing the vials on ice in an open cooler. Temperatures were monitored constantly and held at 2°C-15°C (36°F-59°F).

Room Temperature (Study 2). Samples were reconstituted and left at ~20°C (68°F) and not exposed to UV light.

Elevated Temperature (Study 3). Samples were reconstituted and placed in a darkened incubator set at 37°C (98°F).

Refrigerated Temperature (Study 4). Samples were reconstituted and placed in a refrigerator set at 4°C (39°F) while protected from light.

Freeze/Thaw Cycle (Study 5). Samples were reconstituted, held at -70°C (-94°F) for 2 hours, then thawed to room temperature ~20°C (68°F). Samples were taken for titration at time of complete thaw, then again 1 hour later. Again, the vials were protected from light.

Sampling Technique

Aliquots were drawn from the contents of particular vials at predetermined sampling time points according to the sampling

matrix (Tables 1-5) for each study. Five independent vial titer analyses were then performed for each drawn aliquot at each test session, from which a geometric mean titer (GMT) was calculated. All vials were exposed to the same conditions throughout the study period, but the contents of all of the vials were not tested at every analysis point.

Table 1. Exposure to Sunlight (UV Radiation) at Outside Environmental Temperature 3°C-20°C (37°F-68°F)

	Time (Hours)				
	0	**1**	**2**	**4**	**6**
Vial 1	X		X	X	X
Vial 2	X		X	X	
Vial 3	X	X	X		
Vial 4	X	X			
Reference	X				X

Table 2. Exposure to Room Temperature of ~20°C (~68°F).

	Time (Hours)				
	0	**1**	**2**	**4**	**6**
Vial 1	X		X	X	X
Vial 2	X		X	X	
Vial 3	X	X	X		
Vial 4	X	X			
Reference	X				X

Table 3. Exposure to Elevated Temperature of ~37°C (~98°F).

	Time (Hours)				
	0	**1**	**2**	**4**	**6**
Vial 1	X		X	X	X
Vial 2	X		X	X	
Vial 3	X	X	X		
Vial 4	X	X			
Reference	X				X

Matrices of Aliquots Drawn for Analyses

The measurement assessment was the number of canarypox virus (recombinant) units contained in 1 mL of reconstituted vaccine. Titer analyses were performed using tissue culture evaluated by observing cytopathic effect, which is a standard method for assessing titers in vaccines. The Spearman Karber formula was used to calculate the $\log_{10}$ 50% tissue culture infective dose ($TCID_{50}$)/100 µL, and calculations were made to determine $TCID_{50}$/mL. For a valid test, the titer of the positive reference virus had to be within the established 95% confidence limits, and the negative control wells had to be negative. This is the same method used for potency testing for commercial release of vaccine.

For the first 4 studies, mean titer of the pool was established by taking multiple vials (n = 4) and completing a total of 5 titrations per vial at each time point. Due to the number of time points for each study, the matrix approach (previously described) was used for titers drawn after time point 0. For Study 5, a single vial of vaccine was utilized. At each test time point, the GMT of 5 titrations from each sampled vial was recorded as the titer for that vial. For each environmental condition evaluated, the test time point was compared with the time point 0 (geometric mean of 5 titers generated at reconstitution) of the recombinant canarypox vectored WNV vaccine.

The USDA requires that a pre-determined overage is added to the MPD to assure sufficient vaccine virus is present at the end of shelf life, the so called minimum titer dating (MTD), and an additional overage quantity is added to establish the minimum release titer (MRT). To ensure qualification, vaccines are produced that slightly exceed MRT. A loss of less than 0.5 $\log_{10}$ would keep the vaccine titer well above the USDA MTD. Thus, the tested vaccine would still be expected to perform according to USDA requirements.

Statistical Analysis

All statistical analysis was performed using SAS®-PC version 8.2 (Statistical Analysis Systems Institute, Inc., Cary, NC). Statistical significance was based on 2-

tailed tests in the null hypothesis resulting in *P*-values less than 0.0500. The primary variable was infectious titers expressed as $TCID_{50}$.

Table 4. Exposure to Prolonged Refrigerated Temperature of 2°-7°C (36°-45°F).

	Time (Hours)				
	0	**1**	**2**	**4**	**6**
Vial 1	X		X	X	X
Vial 2	X		X	X	
Vial 3	X	X	X		
Vial 4	X	X			
Reference	X				X

Table 5. Freeze/Thaw of Reconstituted Vial.

			After Vial Thawed	
	Initial Test	**Freeze**	**At Thawing**	**+1 Hour**
Vial 1	X		X	X

RESULTS

Study 1: Evaluation of Virus Titer when Exposed to Sunlight

In the 4 vials of recombinant canarypox vectored vaccine (RECOMBITEK® Equine WNV vaccine) in this study, there were no significant differences observed in starting viral titers at time 0 among the 4 vials ($P > 0.05$). The data generated from this experiment consistently demonstrated that exposure to direct sunlight resulted in a statistically significant ($P < 0.01$) decrease in viral titer levels, approaching or greater than 0.5 $\log_{10}$ at all time points assessed. At the first assessment following exposure to sunlight, the vial titers were approaching or below MTD, and all vial titers were below MTD at later time point assessments.

Study 2: Evaluation of Virus Titer at Room Temperature (20°C [68°F])

In the 4 vials of recombinant canarypox vectored WNV vaccine (RECOMBITEK® Equine WNV vaccine) held protected from direct sunlight at "room temperature" for 8 hours, titer variation throughout the study remained well below the 0.5 $\log_{10}$ benchmark. The maximum negative variation recorded was 0.24 $\log_{10}$ in a single sample. Thus, all GMT assessments were above the MTD, and all but one 4-hour assessment remained above the MRT for the vaccine as well; this same vial was analyzed and exceeded MRT with the next analysis performed at 6 hours. Additionally, there was no statistical difference in titers from time 0 and 6 or 8 hours post-reconstitution.

Study 3: Evaluation of Virus Titer at Elevated Temperatures (37°C [98°F])

In the 4 vials of recombinant canarypox vectored WNV vaccine (RECOMBITEK® Equine WNV vaccine) placed in an incubator at 37°C (98°F; protected from direct sunlight), there was no significant difference observed in starting titers among the 4 vials ($P > 0.05$). All viral titers at all time points, post-reconstitution, were compared to values at time 0, and there were no statistically significant ($P > 0.05$) differences observed at any of the time points in this study. Titer variation remained well below the 0.5 $\log_{10}$ benchmark throughout the study, with the maximum negative variation in titer analysis of 0.18 $\log_{10}$ seen in the analysis performed at 6 hours. All GMT assessments exceeded MTD and MRT standards for the vaccine throughout the study.

Study 4: Evaluation of Virus Titer Under Refrigeration (2°C to 7°C [36°F to 45°F])

Titer variation remained well below the 0.5 $\log_{10}$ benchmark at all measured time points throughout the study when recombinant canarypox vectored WNV vaccine (RECOMBITEK® Equine WNV vaccine) was refrigerated at 4°C [39°F] for 30 hours and protected from direct sunlight. All GMT assessments exceeded MTD requirements throughout the study, and GMT assessments at 24 and 30 hours exceeded MRT requirements for the vaccine. The maximum negative variation in titer was 0.28 $\log_{10}$ at 8 hours in a single sample, which is within the variability of the testing method used.

Study 5: Evaluation of Virus Titer With a Freeze/Thaw Cycle

Recombinant canarypox vectored WNV vaccine (RECOMBITEK® Equine WNV vaccine) was frozen at -70°C [-94°F] for 2 hours, then thawed and held at room temperature for 1 hour. The vial was protected from exposure to direct sunlight throughout the process. Aliquots were drawn from the contents of the vial at the time of thawing and 1 hour after the product was thawed, and titers performed at both of those times. Titers generated from this experiment were analyzed using a 1-way analysis of variance (ANOVA) with time as the factor. The results of the 1-way ANOVA showed that the titer of the vial did not significantly change over the time period of testing ($P > 0.05$). All GMT assessments exceeded both MTD and MRT standards for the vaccine throughout the study.

DISCUSSION

Field conditions of equine practice often create situations in which vaccines may not be able to be used precisely according to label recommendations. In order to determine how some of these factors might affect the product stability, the effects of some environmental conditions on a commercial recombinant WNV vaccine stability were analyzed.

Each dose of live vaccine released to the field has 2 built-in safety margins to ensure that a sufficient quantity of virus is present in vaccine to protect horses under practical working conditions. In addition, manufacturing, to ensure compliance, adds an additional safety margin to ensure consistent release of the product. In the present study, the goal was to ensure that under the conditions tested, sufficient quantity of the virus was remained to meet the qualifications of the MTD. The MTD is higher than the MPD, at which the efficacy of the vaccine was demonstrated.

In all 5 of these stability studies, the vaccine was considered within specifications if titer-loss was less than 0.5 $\log_{10}$ as compared to time point 0, which would still allow the vaccine vial titer to be well above the USDA MTD. Thus, the tested vaccine would still be expected to perform to USDA requirements for the vaccination of horses against West Nile virus. In Study 1, when the vaccine was exposed to UV irradiation (sunlight), the vial titer variation approached or was greater than 0.5 $\log_{10}$ at all test points. Therefore, vaccine exposed to direct sunlight for 1, 2, 4, or 6 hours should not be considered for use and should be disposed of appropriately.

In order to critically assess the measurements and this vaccine, the investigators gave greater weight to lower measured titer values by assessing the average titer for each time point using geometric means rather than arithmetic means. This method resulted in a more conservative estimate of titer levels for each specific time point. This conservative approach better serves the practitioner by essentially measuring a worst-case scenario impact of exposure to environmental conditions over time.

These data were developed to provide useful information to equine veterinary practitioners about the impact of various environmental conditions on the stability of RECOMBITEK® Equine WNV vaccine. The results clearly reinforce the need to protect reconstituted RECOMBITEK® Equine WNV vaccine from direct sunlight. These studies also indicate that it is reasonable to expect the vaccine to remain viable under the following conditions, when protected from direct sunlight:

- Up to 8 hours following reconstitution when kept at room temperature
- Up to 30 hours following reconstitution when kept refrigerated
- Up to 6 hours when kept at elevated environmental temperatures (37°C [98°F])
- At thawing and up to 1 hour after thaw ing, in event the vaccine should be frozen

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REFERENCES

1. Minke JM, Siger L, Karaca K, et al: Recombinant canarypox virus vaccine carrying the prM/E genes of West Nile virus protects horses against a West Nile virus-mosquito challenge. *Arch Virol* 2004;18(Suppl):221-230.
2. Siger L, Bowen R, Karaca K, et al: Assessment of the efficacy of a single dose of a recombinant vaccine against West Nile virus in response to natural challenge with West Nile virus-infected mosquitoes in horses. *Am J Vet Res* 2004;65,1459-1462.