Summary of Studies Supporting USDA Product Licensure

Establishment Name	Boehringer Ingelheim Animal Health USA Inc.
USDA Vet Biologics Establishment Number	124
Product Code	48W5.20
True Name	Encephalomyelitis-West Nile Virus Vaccine, Eastern & Western & Venezuelan, Killed Virus, Tetanus Toxoid
Tradename(s) / Distributor or Subsidiary (if different from manufacturer)	Vetera VEWT + WNV - No distributor specified
Date of Compilation Summary	February 06, 2019

Disclaimer: Do not use the following studies to compare one product to another. Slight differences in study design and execution can render the comparisons meaningless.

Study Type	Efficacy
Pertaining to	Clostridium tetanus
Study Purpose	Demonstration of efficacy against Clostridium tetanus
Product Administration	One dose, administered intramuscularly
Study Animals	10 guinea pigs (10 vaccinates)
Challenge Description	Not applicable
Interval observed after challenge	Not applicable
Results	Vaccinate serum samples were collected and pooled, then tested for antitoxin content by indirect Enzyme-Linked Immunosorbent Assay. A satisfactory value which met the requirements per 9 CFR 113.114(c) was achieved.
USDA Approval Date	April 18, 2008

Study Type	Efficacy
Pertaining to	Eastern equine encephalomyelitis
Study Purpose	Demonstration of efficacy against Eastern equine encephalomyelitis
Product Administration	Two doses, administered intramuscularly, 14 to 21 days apart
Study Animals	12 guinea pigs (10 vaccinates, 2 controls)
Challenge Description	Not applicable
Interval observed after challenge	Not applicable
Results	Serum samples were tested by a plaque reduction, serum neutralization test, 14 days after the second injection. Vaccinates and controls were evaluated in terms of Eastern equine encephalomyelitis per the criteria in 9 CFR 113.207(b) and the requirements were met.
USDA Approval Date	April 18, 2008

Study Type	Efficacy
Pertaining to	Venezuelan equine encephalomyelitis
Study Purpose	Demonstration of efficacy against Venezuelan Equine Encephalomyelitis
Product Administration	Two doses, administered intramuscularly, 14 to 21 days apart
Study Animals	12 guinea pigs (10 vaccinates, 2 controls)
Challenge Description	Not applicable
Interval observed after challenge	Not applicable
Results	Serum samples were tested by a plaque reduction, serum neutralization test, 14 days after the second injection. Vaccinates and controls were evaluated in terms of Venezuelan equine encephalomyelitis per the criteria in 9 CFR 113.207(b) and the requirements were met.
USDA Approval Date	April 18, 2008

Study Type	Efficacy
Pertaining to	Western equine encephalomyelitis
Study Purpose	Demonstration of efficacy against Western equine encephalomyelitis
Product Administration	Two doses, administered intramuscularly, 14 to 21 days apart
Study Animals	12 guinea pigs (10 vaccinates, 2 controls)
Challenge Description	Not applicable
Interval observed after challenge	Not applicable
Results	Serum samples were tested by a plaque reduction, serum neutralization test, 14 days after the second injection. Vaccinates and controls were evaluated in terms of Western equine encephalomyelitis per the criteria in 9 CFR 113.207(b) and the requirements were met.
USDA Approval Date	April 18, 2008

<table>
<tr><td>Study Type</td><td>Efficacy</td></tr>
<tr><td>Pertaining to</td><td>West Nile Virus (WNV)</td></tr>
<tr><td>Study Purpose</td><td>Demonstration of twelve month duration of immunity against disease caused by WNV</td></tr>
<tr><td>Product Administration</td><td>Two doses, administered intramuscularly, 25 days apart</td></tr>
<tr><td>Study Animals</td><td>30 horses (20 vaccinates, 10 placebo controls) 4-5 months of age</td></tr>
<tr><td>Challenge Description</td><td>West Nile Virus was administered at 380 days (10 vaccinated and 5 placebo control animals) or 408 days (10 vaccinated and 5 placebo control animals) post-final vaccination.</td></tr>
<tr><td>Interval observed after challenge</td><td>Horses were observed twice daily for 14 days post-challenge and once daily for an additional 7 days post-challenge.</td></tr>
<tr><td>Results</td><td>An animal was considered affected by challenge if it developed neurological disease, as measured by mortality and microscopic evidence of virus-induced brain disease (histopathology).

Animals were also monitored for viremia (detection of WNV in the blood).

Results are summarized as follows:
<table>
<tr><th>Outcome</th><th>Controls</th><th>Vaccinates</th></tr>
<tr><td>Mortality</td><td>7/10 (70%)</td><td>1/20 (5%)</td></tr>
<tr><td>Viremia at least one day</td><td>10/10 (100%)</td><td>2/20 (10%)</td></tr>
</table>

See raw data on following pages.</td></tr>
<tr><td>USDA Approval Date</td><td>September 3, 2010</td></tr>
</table>

Treatment	#	Died or Euthanized due to disease severity	Severity Histopathological lesions	
			Medulla	Pons
Controls (10 horses)	1	Yes	3	3
	2	Yes	3	3
	3	Yes	3	3
	4	Yes	3	3
	5	Yes	3	3
	6	Yes	2	2
	7	Yes	1	1
	8	No	1	1
	9	No	1	1
	10	No	1	0.5
Vaccinates (20 horses)	1	Yes	3	3
	2	No	2	0.5
	3	No	1	1
	4	No	1	0.5
	5	No	1	0.5
	6	No	1	0.5
	7	No	0.5	0.5
	8	No	0.5	0.5
	9	No	0.5	0
	10	No	0	0.5
	11	No	0	0
	12	No	0	0
	13	No	0	0
	14	No	0	0
	15	No	0	0
	16	No	0	0
	17	No	0	0
	18	No	0	0
	19	No	0	0
	20	No	0	0

Scoring of histopathological lesions:	
0 =	No significant lesions.
0.5 =	Rare, small, multifocal glial nodules scattered throughout the parenchyma.
1 =	Mild, nonsuppurative encephalitis characterized by mild multifocal perivascular cuffs with lymphocytes and plasma cells and a rare neutrophil and scattered multifocal glial nodules composed of glial cells with a few mononuclear inflammatory cells. Occasionally within this grade, there may be minimval perivascular cuffing and more moderate scattered glial nodules.
2 =	Moderate nonsuppurative encephalitis characterized by moderate lymphoplasmacytic perivascular cuffs around many vessels and multifocal accumulations of glial nodules scattered throughout the parenchyma.
3 =	Severe nonsuppurative encephalitis characterized by severe and thick lymphoplasmacytic perivascular cuffing with multiple scattered glial nodules throughout the parenchyma.

Viremia: Days Post-challenge

Treatment	#	0	1		2		3		4		5		6		7	8	9	10	14	21
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM						
Controls (10 horses)	1				15	25	10				5								D	D
	2					30		5	20		5								D	D
	3				25	470	160	210	175	75	100							D	D	D
	4			5	20	205	175	90	75	50								D	D	D
	5			40	130	30	25	50	10									D	D	D
	6				165	110	65	55	10										D	D
	7			10	330	200	180	225	115	15									N	D
	8				50	90	35	105	20	10										
	9			5	30	95	25	135	240	40	20									
	10					80	70	40	10											
Vaccinates (20 horses)	1																		D	D
	2																			
	3																			
	4																			
	5																			
	6				95															
	7																			
	8				40															
	9																			
	10																			
	11																			
	12																			
	13																			
	14																			
	15																			
	16																			
	17																			
	18																			
	19																			
	20																			

Actual value in plaque-forming units per milliliter equivalents (PFUeq/mL) = Positive for virus isolation
Blank = Negative for virus isolation (<5 PFUeq/mL)
D = Dead
N = Not recorded; horse was circling with sporadic head / neck tremors.

<table>
<tr><td>Study Type</td><td>Efficacy</td></tr>
<tr><td>Pertaining to</td><td>West Nile Virus</td></tr>
<tr><td>Study Purpose</td><td>Demonstration of efficacy against WNV</td></tr>
<tr><td>Product Administration</td><td>Two doses, administered intramuscularly 21 days apart</td></tr>
<tr><td>Study Animals</td><td>28 horses (19 vaccinates, 9 placebo controls) 4-5 months of age</td></tr>
<tr><td>Challenge Description</td><td>West Nile Virus was administered intrathecally at 14 days (to 10 vaccinated and 5 placebo control animals) and 28 days (to 9 vaccinated and 4 placebo control animals) after the second vaccination</td></tr>
<tr><td>Interval observed after challenge</td><td>Horses were bled on the day of challenge, twice daily for 6 days post-challenge, once daily for an additional 4 days post-challenge, and on day 14 post-challenge</td></tr>
<tr><td>Results</td><td>The primary outcome was viremia (detection of WNV in the blood). While the test method was quantitative, an animal was considered to be positive (affected by challenge) if any virus was detected in the blood on one or more occasions post-challenge.

The number of animals positive for (affected by) viremia at least once is summarized as follows:
<table><tr><th>Controls</th><th>Vaccinates</th></tr><tr><td>8/9 (89%)</td><td>1/19 (5%)</td></tr></table>
See raw data on the following page.</td></tr>
<tr><td>USDA Approval Date</td><td>August 25, 2008</td></tr>
</table>

Viremia: Days Post-Challenge

Treatment	Horse ID	0	1		2		3		4		5		6		7	8	9	10	14
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM					
Controls (9 horses)	**13**		15	20	390	280	135			20									
	19			5		40			5	20	65	45							
	20			125	1475	645	355	495	120	15									
	45			20	85	235	140	235	145	80	15								
	67				165														
	71				110	675	110	70	120	70									
	72																		D
	74				5	60	30	15	20	15		10							
	79				10	15	20		15		10	5							
Vaccinates (19 horses)	**14**																		
	16																		
	21																		
	22																		
	23																		
	24																		
	25																		
	26																		
	27																		
	37																		
	73																		
	75																		
	77																		
	80																		
	81																		
	82																		
	83																		
	84																		
	85				5		5		5										

Actual value in plaque-forming units per milliliter equivalents (PFUeq/mL) = Positive for virus isolation
Blank = Negative for virus isolation (<5 PFUeq/mL)
D = Dead (euthanized on Day 11 due to West Nile Virus)

<table>
<tr><td>Study Type</td><td>Efficacy</td></tr>
<tr><td>Pertaining to</td><td>West Nile Virus (WNV)</td></tr>
<tr><td>Study Purpose</td><td>Demonstration of six month duration of immunity against WNV</td></tr>
<tr><td>Product Administration</td><td>Two doses, administered intramuscularly 21 days apart</td></tr>
<tr><td>Study Animals</td><td>30 horses (20 vaccinates, 10 placebo controls) 4-5 months of age</td></tr>
<tr><td>Challenge Description</td><td>Challenge by West Nile Virus was administered at 180 days (Challenge Group 1: 10 vaccinated and 5 placebo control animals) or 243 days (Challenge Group 2: 10 vaccinated and 5 placebo control animals) after second vaccination</td></tr>
<tr><td>Interval observed after challenge</td><td>Horses were bled on the day of challenge, twice daily for 6 days post-challenge, once daily for an additional 4 days post-challenge, and on day 14 post-challenge</td></tr>
<tr><td>Results</td><td>The primary outcome was viremia (detection of WNV in the blood). While the test method was quantitative, an animal was considered to be positive (affected by challenge) if any virus was detected in the blood on one or more occasions post-challenge.

The number of animals positive for viremia at least once (affected) is summarized as follows:
<table>
<tr><th>Challenge Group</th><th>Controls</th><th>Vaccinates</th></tr>
<tr><td>1</td><td>5/5 (100%)</td><td>2/10 (20%)</td></tr>
<tr><td>2</td><td>5/5 (100%)</td><td>4/10 (40%)</td></tr>
<tr><td>Combined</td><td>10/10 (100%)</td><td>6/20 (30%)</td></tr>
</table>

See raw data on the following page.</td></tr>
<tr><td>USDA Approval Date</td><td>October 21, 2009</td></tr>
</table>

Viremia: Days Post-challenge

Treatment	Horse ID	0	1		2		3		4		5		6		7	8	9	10	14	Final Outcome
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM						
Controls (5 horses) Challenge 1	S2				170	170	165	55	105	45										Positive
	S4				425	20	30	40	85	60	65								N	Positive
	S10				10	300	125	125	80	45										Positive
	S11					50	30	40	40	25										Positive
	S13					410	110	135	110	55	15								N	Positive
Vaccinates (10 horses) Challenge 1	S1																			Negative
	S3																			Negative
	S5																			Negative
	S6																			Negative
	S7				470		45	5												Positive
	S8				15															Positive
	S9																		N	Negative
	S12																			Negative
	S14																			Negative
	S15																			Negative
Controls (5 horses) Challenge 2	S50			5	535	500	80	100	50	10										Positive
	S53			20	320	380	100	135	45	10										Positive
	S54								5		5	5	5						N	Positive
	S55		10	5	95	70	30	25	40											Positive
	S59				90	265	20	70	45	45	5									Positive
Vaccinates (10 horses) Challenge 2	S46																			Negative
	S47								5											Positive
	S48																			Negative
	S49				15															Positive
	S51																			Negative
	S52																			Negative
	S56																			Negative
	S57				5	5														Positive
	S58			5																Positive
	S60																			Negative

Actual value in plaque-forming units per milliliter equivalents (PFU eq/mL) = Positive for virus isolation
Blank = Negative for virus isolation (<5 PFU eq/mL)
N = Not recorded
Positive = affected by challenge if virus was detected in the blood on one or more occasions post-challenge.
Negative = virus was detected in the blood on zero occasions post-challenge.

Study Type	Safety
Pertaining to	All fractions
Study Purpose	To demonstrate safety in pregnant mares under field conditions at two different test sites
Product Administration	Two intramuscular doses, given 16-28 days apart. 54 pregnant mares were injected with placebo and 325 pregnant mares were vaccinated with test product.
Study Animals	Three hundred seventy-nine pregnant mares at two locations were included in the study. The mares were confirmed to be pregnant by serum hormonal evaluation on the day of the first vaccination.
Challenge Description	Not applicable
Interval observed after vaccination	1st and 2nd trimester: Mares observed immediately after vaccination and daily for overall health and for abortion. Resulting foals were observed daily for 7 days following birth. 3rd trimester: Mares observed immediately after vaccination and daily for overall health and for abortion. Resulting foals were observed daily for 30 days following birth.
Results	Results shown on next page

Results

Study 2013-PM-1009
North Dakota Site:

Group	Vaccinated	Confirmed Pregnant	Foals	Parturition Rate
1st trimester/ product	143	127	114	90%
1st trimester/ placebo	59	54	49	91%
2nd trimester/ product	6	6	6	100%
3rd trimester/ product	140	117	117	100%
Total – all animals	**348**	**304**	**286**	**94%**
Total – product only	**289**	**250**	**237**	**95%**
Total – placebo only	**59**	**54**	**49**	**91%**

Study 2013-PM-1009
Misssouri Site:

Group	Vaccinated	Confirmed Pregnant	Foals	Parturition Rate
2011 3rd trimester	5	5	5	100%
2012 1st trimester	1	1	1	100%
2012 2nd trimester	53	43	39	91%
2012 3rd trimester	26	26	25	96%
Total – product	**85**	**75**	**70**	**93%**

Study 2014-PM-1009
North Dakota Site:

Group	Vaccinated	Confirmed Pregnant	Foaled	Parturition Rate	Foals Survived to End of Observation Period
2nd trimester vaccinated	52	52	52	100%	51*
3rd trimester vaccinated	69	69	67**	97.1%	67

*Lost foal affirmed by study cooperator to be due to causes other than vaccination.
**One mare died due to causes other than vaccination, as affirmed by study cooperator.
All other foals were normal and healthy

USDA Approval Date	September 12, 2014

<table>
<tr><td>Study Type</td><td>Safety</td></tr>
<tr><td>Pertaining to</td><td>All fractions</td></tr>
<tr><td>Study Purpose</td><td>To demonstrate safety under field conditions</td></tr>
<tr><td>Product Administration</td><td>Two doses, administered intramuscularly 3 – 4 weeks apart</td></tr>
<tr><td>Study Animals</td><td>556 horses, including 438 foals between 2 months and approximately 1 year of age</td></tr>
<tr><td>Challenge Description</td><td>Not applicable</td></tr>
<tr><td>Interval observed after challenge</td><td>Not applicable</td></tr>
<tr><td>Results</td><td>Horses were observed at least daily following each vaccination, until resolution of any observed reactions. Observations ended 14 days after the second vaccination.

There were no systemic reactions observed at any of the sites. One horse died from causes affirmed by licensee not associated with vaccination.

Scoring Method for Injection Site Reactions:
0 = No reaction
1 = Localized swelling at or near the injection site which is not visible; detectable only by palpation. Not painful.
2 = Localized visible swelling at or near the injection site. Not painful.
3 = Localized visible swelling at or near the injection site. Raised, circumscribed and painful when palpated.

Local injection site reactions are summarized below across the sites:
<table>
<tr><th rowspan="2">Site</th><th rowspan="2">Total Number Of Vaccinates</th><th rowspan="2">Number Of Vaccinates Administered 2 doses</th><th colspan="2">Vaccinates With Transient Injection Site Swelling</th><th colspan="2">Number Of Normal Vaccinates</th></tr>
<tr><td>After 1st dose</td><td>After 2nd dose</td><td>After 1st dose</td><td>After 2nd dose</td></tr>
<tr><td>Missouri</td><td>315</td><td>314</td><td>3 (1.0%)</td><td>9 (2.9%)</td><td>312 (99.0%)</td><td>305 (97.1%)</td></tr>
<tr><td>Oklahoma</td><td>110</td><td>110</td><td>1 (0.9%)</td><td>2 (1.8%)</td><td>109 (99.1%)</td><td>108 (98.2%)</td></tr>
<tr><td>Texas</td><td>131</td><td>131</td><td>0 (0%)</td><td>0 (0%)</td><td>131 (100%)</td><td>131 (100%)</td></tr>
<tr><td>Total</td><td>556</td><td>555</td><td>4 (0.7%)</td><td>11 (2.0%)</td><td>552 (99.3%)</td><td>544 (98.0%)</td></tr>
</table>

Results from each site are summarized on the following page.</td></tr>
</table>

Missouri Site:

Summary	Number Of Vaccinates	Number Of Vaccinates Administered 2 doses	Vaccinates With Transient Injection Site Swelling		Number Of Normal Vaccinates	
Age			After 1st dose	After 2nd dose	After 1st dose	After 2nd dose
2-4 months	55	55	0	0	55	55
5-7 months	8	8	0	0	8	8
8-11 months	1	1	0	0	1	1
1 year	170	170	1	2	169	168
≥ 2 years	81	80	2	7	79	73
Total	**315**	**314**	**3**	**9**	**312**	**305**

Horse No.	Age	Reaction Description	Injection #	Day	Score	Resolution Day
10	11 y	Swelling on day 3, 5.5 cm x 2.25 cm x 5mm	2	3	2	7
22	8 y	Swelling on day 3, 12 cm circle, raised 1.5 cm, painful,	2	3	3	7
129	1 y	Swelling on day 3, 2.3 cm circle, raised 4 mm, painful but no heat	2	3	3	7
183	1 y	Swelling on day 7, raised lesion 1.5 cm circle, height 0.2 cm	1	7	2	14
183	1 y	Swelling on day 1, 3 cm lesion, not raised but palpalble	2	1	1	3
222	9 y	Swelling on day 3, 6 cm x 7 cm x 1.2 cm, raised lesion hard and painful	2	3	3	7
266	10 y	Swelling localized in several places unsure if related to vaccine	1	1	2	3
266	10 y	Swelling small palpapable mass ~ 2 cm size, still present day 3 no worse	2	1	2	3
271	13 y	Swelling 5cm circle, raised 5 mm, solid and painful	2	3	3	7
288	8 y	Swelling < 2 cm, raised lesion ~ 1 mm deep	1	3	2	7
288	8 y	Swelling ~ 8.5 cm circle raised ~ 1.3 cm, painful, not hot to touch	2	3	3	7
300	10 y	Swelling 6 cm circle, solid swelling not painful	2	3	2	7

Oklahoma Site:

Summary	Number Of Vaccinates	Number Of Vaccinates Administered 2 doses	Vaccinates With Transient Injection Site Swelling		Number Of Normal Vaccinates	
Age			After 1st dose	After 2nd dose	After 1st dose	After 2nd dose
4-6 months	49	49	1	1	48	48
1 year	25	25	0	1	25	24
≥ 2 years	36	36	0	0	36	36
Total	**110**	**110**	**1**	**2**	**109**	**108**

Horse No.	Age	Reaction Description	Injection #	Day	Score	Resolution Day
19-A	4 m	Swelling redness painful injection area 6 cm in diameter, reaction subsided in 10 days	1	7	3	17
33-A	5 m	Small swelling, 3 cm diameter, subsided in 3 days	2	1	2	4
43	1 y	Mid-sized swelling, 5 cm diameter, reduced to 2.5 cm in 6 days; small, hard 2 cm at 10 days, probable subcutaneous leakage	2	1	2	Study End

Texas Site:

Summary	Number Of Vaccinates	Number Of Vaccinates Administered 2 doses	Vaccinates With Transient Injection Site Swelling		Number Of Normal Vaccinates	
Age			After 1st dose	After 2nd dose	After 1st dose	After 2nd dose
7-9 months	130	130	0	0	130	130
≥ 2 years	1	1	0	0	1	1
Total	**131**	**131**	**0**	**0**	**131**	**131**

USDA Approval Date	September 14, 2009