EQUINE

Evaluation of administration of West Nile virus vaccine to pregnant broodmares

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Objective—To determine whether administration of killed West Nile virus vaccine was associated with pregnancy loss among broodmares.

Design—Retrospective cohort study.

Animals—595 mares.

Procedure—Records of pregnant mares with known vaccination history from 4 farms were reviewed. Information obtained from 595 mares included mare's identification; farm; age; breed; reproductive status; last breeding date; date last known pregnant; vaccination date; age of conceptus at vaccination; vaccination during the early embryonic, early fetal, and late fetal periods; and whether an early embryonic death (EED), early fetal loss (EFL), or late fetal loss (LFL) occurred. The relationships between the dichotomous outcomes of loss (eg, EED, EFL, LFL) and independent categoric variables (eg, vaccination during the early embryonic, early fetal, or late fetal periods) were examined.

Results—Vaccination of pregnant mares during any period of gestation was not associated with increased incidence of pregnancy loss.

Conclusions and Clinical Relevance—Many mares are already pregnant at the onset of mosquito season, when mares are more likely to be vaccinated than at other times. Our findings provide evidence that vaccine administration will not compromise pregnancy in horses. (*J Am Vet Med Assoc* 2004;225:1894–1897)

Some public controversy exists regarding the safety of administration of killed **West Nile virus (WNV)** vaccine[a] to pregnant broodmares.[1,2] Although the vaccine is not labeled for use in pregnant mares, the USDA Animal and Plant Health Inspection Service stated in a 2003 press release that no evidence was available that would support a claim that administration of killed WNV vaccine adversely affected pregnancies in mares[1] and suggested that pregnancy loss rates in vaccinated mares were similar to those typically reported in the equine breeding industry.[1,3] To the authors' knowledge, no data have been published that would allow practitioners to evaluate the frequency or probability of reproductive losses as a result of administration of this vaccine to mares. The purpose of the study reported here was to determine whether administration of WNV vaccine to pregnant mares was associated with pregnancy loss.

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Materials and Methods

Study population—Mares from 4 sources were used in the study: a Quarter Horse-type breeding facility in Texas for which certain authors (DJV, CJB, and TLB) provided equine reproductive services; a large Quarter Horse breeding farm in Texas for which one of the authors (GPB) provided equine reproductive services; a Thoroughbred farm in central Kentucky for which one of the authors (JPM) provided reproductive services; and farms in central Kentucky with Thoroughbred broodmares that received reproductive services from the private equine practice of one of the authors (JPM). These sources were selected because of their willingness to provide data, quality of mare health record keeping, and frequency of reproductive examinations during pregnancy. For purposes of this report, these 4 sources will hereafter be referred to as farms. During 2003, all mares determined to be pregnant on days 14 to 16 of gestation from these 4 farms were eligible for inclusion; mares that did not become pregnant were excluded from the study.

The health records for eligible mares at each farm were reviewed to extract the following data: farm; age; reproductive status of mare (maiden [ie, never bred], foaling in 2003, barren [ie, bred without conception], lost previous pregnancy, or not bred in 2002); last breeding date; date and gestational age at time of vaccination; date and gestational age at time last examined and found to be pregnant; outcome (pregnancy loss or no pregnancy loss); and date and gestational age of conceptus at time pregnancy loss was detected. Because examinations of pregnancy status were irregular and less frequent during the early fetal and late fetal periods, these data often spanned a time interval between the date the mare was last determined to be pregnant and the date the mare was determined to no longer be pregnant. All mares included in the study had received a primary vaccination and a booster vaccination against WNV in 2002 and, when applicable, additional routine vaccinations prior to inclusion in the study (ie, all mares were vaccinated according to the manufacturer's recommendations for primary and annual vaccination prior to inclusion in the study).

Because the risk of pregnancy loss is known to vary during gestation[4,5] and because a cohort of nonvaccinated mares was not available for a clinical trial with random assignment of vaccination, pregnancy losses were compared between pregnant mares that were or were not administered vaccine during specified periods of pregnancy. For purposes of this study, 3 periods of pregnancy were defined: the **early embryonic (EE)** period (from ovulation to 40 days of gestation), the **early fetal (EF)** period (41 to 150 days of gestation), and the **late fetal (LF)** period (> 150 days of gestation; including stillbirths). Pregnancy losses were categorized according to gestational age of the embryo or fetus by use of the following definitions: **early embryonic death** (**EED**; pregnancy loss prior to day 40 of gestation), **early fetal loss** (**EFL**; pregnancy loss between 41 and 150 days of gestation), and **late fetal loss** (**LFL**; pregnancy loss at > 150 days of gestation). The frequency of EED and EFL were compared between mares that were pregnant and vaccinated during the EE period and mares that were pregnant but not vaccinated during the EE period. The frequency of EFL was compared between mares that were pregnant and vaccinated during the EF period and mares that were pregnant but not vaccinated during the EF period. The frequency of LFL was compared between mares that were pregnant and vaccinated during the LF period and mares that were pregnant but not vaccinated during the LF period.

Statistical analyses—Data were summarized via contingency tables for categoric variables and medians and ranges for continuous data. The relationships between each of the dichotomous outcomes of loss (eg, EED, EFL, or LFL) and independent categoric variables (eg, vaccination during the EE, EF, or LF period) were examined by use of 2-way and multiway contingency tables; the χ^2 and Fisher exact tests were used to compare proportions. The relationships between pregnancy outcomes (eg, EED, EFL, or LFL) and continuous variables (eg, age) were examined by use of the Wilcoxon rank sum test (for dichotomous categoric variables) or the Kruskal-Wallis test (for polytomous categoric variables such as farm). Post hoc testing for polytomous comparisons was performed by use of a modification of the method of Scheffé. The association of the dichotomous outcomes (eg, EFL) and multiple dependent factors (ie, multivariate analysis) was assessed by use of multivariable logistic regression. The principal aim of this study was to assess the association of vaccination with pregnancy loss (ie, EED, EFL, or LFL). Variables that were significantly associated with either pregnancy loss or vaccination were included in multivariate analysis to adjust for potential confounding and to obtain estimates of the association of vaccination with pregnancy loss adjusted for other factors significantly associated with loss. Data analyses were conducted with commercial software.[b] A significance level of $P < 0.05$ was used for all analyses.

Results

Data were collected from 595 mares that were determined to be pregnant at 14 to 16 days after ovulation. There were 175 mares from farm 1, 158 mares from farm 2, 137 mares from farm 3, and 125 mares from farm 4. Age was known for 481 mares and ranged from 2 to 25 years (median, 9 years). Reproductive status was known for 480 mares; 69 were maidens, 345 foaled, 27 were barren, 34 had lost a previous pregnancy, and 5 were not bred. One hundred ninety-four mares were vaccinated once or more during the EE period, 356 mares were vaccinated once or more during the EF period, and 106 mares were vaccinated once during the LF period. Overall incidences of EED, EFL, LFL, and total pregnancy losses were 31 of 595 (5.2% [P= 0.375]), 20 of 564 (3.5% [P = 0.109]), 6 of 544 (1.1% [P = 0.865]), and 57 of 595 (9.6%), respectively.

Association of pregnancy loss with vaccination during the EE period—Of the 595 pregnant mares, 31 (5.2%) had EED. Of these 595 mares, 194 mares (32.6%) were vaccinated during the EE period (P < 0.001); 7 of these mares were vaccinated twice during the EE period. Incidence of EED was not significantly greater among mares vaccinated during the EE period (9/194 [4.6%]) than among mares not vaccinated during the EE period (22/401 [5.5%]). Median gestational age at the time of first vaccination was 21 days (range, 0 to 31 days [0 = day of ovulation]). Median gestational age at time of diagnosis of EED was 29 days (range, 16 to 40 days). Median time from the first vaccination during the EE period to EED was 19 days (range, 8 to 28 days). None of the horses vaccinated a second time during the EE period sustained an EED. The incidence of EFL was not significantly greater for mares vaccinated during the EE period (10/185 [5.4%]) than for mares not vaccinated during the EE period (10/371 [2.7%]). There was no significant difference in the incidence of mares with either an EED or an EFL among those that were vaccinated during the EE period (19/194 [9.8%]) and mares that were not vaccinated during the EE period (32/401 [8.0%]).

Association of pregnancy loss with vaccination during the EF period—Of the 564 mares that were pregnant at the onset of the EF period (ie, 595 mares minus 31 mares that had EED), 20 (3.5%) had EFL. Of these 564 mares, 356 (63.1%) were vaccinated during the EF period (P < 0.001); 38 (6.7%) were vaccinated twice during the EF period. The incidence of EFL in mares vaccinated during the EF period (4/356 [1.1%]) was significantly (P < 0.001) less than in mares not vaccinated during the EF period (16/208 [7.7%]). Median gestational age at the time of first vaccination during the EF period was 93 days (range, 41 to 130 days). Median gestational age at time of diagnosis of EFL was 60 days (range, 42 to 130 days). Median time from the first vaccination during the EF period to EFL was 17 days (range, 8 to 25 days). None of the horses vaccinated a second time during the EF period had an EFL.

Association of pregnancy loss with vaccination during the LF period—Of the 544 mares that were pregnant at the onset of the LF period (ie, 595 mares minus 31 mares that had EED minus 20 mares that had EFL), 25 (4.6%) had an LFL. Of these 544 mares, vaccination status during the LF period was known for 542. Of the 542 mares, 106 (19.6%) were vaccinated during the LF period (P < 0.001); none were vaccinated twice during the LF period. The incidence of LFL in mares vaccinated during the LF period (6/106 [5.7%]) was not significantly different than in mares not vaccinated during the LF period (19/436 [4.4%]). Median gestational age at the time of first vaccination during the LF period was 178 days (range, 151 to 223 days). Median gestational age at time of diagnosis of LFL was 261 days (range, 158 to 356 days); these data included 5 stillborn foals. Excluding the 5 stillborn foals, median gestational age at time of diagnosis of LFL was 243 days (range, 158 to 330 days). Median time from vaccination during the LF period to LFL was 79 days (range, 50 to 194 days). None of the horses was vaccinated a second time during the LF period.

Association of farm with pregnancy loss and with vaccination—There were no significant differences among farms in the frequency of EED, EFL, or LFL by farms (**Table 1**). There were significant differences among farms in the proportion of vaccinated mares during each of the specified periods of pregnancy (**Table 2**).

Table 1—Pregnancy losses (No. [%]) on 4 farms among 595 mares known to be pregnant during 2003.

Fetal loss type	Farm 1	Farm 2	Farm 3	Farm 4	Total
No EED	120 (96)	169 (97)	148 (94)	127 (93)	**564 (95)**
EED	5 (4)	6 (3)	10 (6)	10 (7)	**31 (5)**
Total	**125**	**175**	**158**	**137**	**595 (100)**
No EFL	117 (97)	165 (98)	138 (93)	124 (98)	**544 (96)**
EFL	3 (2)	4 (2)	10 (7)	3 (2)	**20 (4)**
Total	**120**	**169**	**148**	**127**	**564 (100)**
No LFL	111 (95)	159 (96)	131 (95)	117 (94)	**537 (99)**
LFL	6 (5)	6 (4)	7 (5)	7 (6)	**7 (1)**
Total	**117**	**165**	**138**	**124**	**544 (100)**

EED = Early embryonic death. EFL = Early fetal loss. LFL = Late fetal loss.

Table 2—Vaccination period for West Nile virus on 4 farms among 595 mares (No. [%]) known to be pregnant during 2003.

Vaccination period	Farm 1	Farm 2	Farm 3	Farm 4	Total
Early embryonic					
No	115 (92)	127 (73)	71 (45)	88 (64)	**401 (67)**
Yes	10 (8)	48 (27)	87 (55)	49 (36)	**194 (33)**
Total	**125**	**175**	**158**	**137**	**595 (100)**
Early fetal					
No	26 (22)	49 (29)	16 (11)	117 (92)	**208 (37)**
Yes	94 (78)	120 (71)	132 (89)	10 (8)	**356 (63)**
Total	**120**	**169**	**148**	**127**	**564 (100)**
Late fetal					
No	106 (91)	143 (87)	63 (46)	124 (100)	**436 (80)**
Yes	11 (9)	21 (13)	74 (54)	0	**106 (20)**
Total	**117**	**164**	**137**	**124**	**542 (100)**

Association of age of mare with pregnancy loss and with vaccination—There were no significant differences in age between mares that had an EED (median, 10 years; range, 2 to 25 years) and mares that did not have an EED (median, 9 years; range, 3 to 23 years) or between mares vaccinated during the EE period (median, 9 years; range, 2 to 25 years) and mares not vaccinated during the EE period (median, 9 years; range, 3 to 23 years). Among mares pregnant during the EF period, there were no significant differences in age between mares that had an EFL and mares that did not or between mares vaccinated during the EF period and mares that were not vaccinated during the EF period. Similarly, among mares pregnant during the LF period, there were no significant differences in age between mares that had an LFL and mares that did not or between mares vaccinated during the LF period and mares not vaccinated during this period. Ages of mares were similar among mares from farm 1 (median, 9 years; range, 4 to 23 years), farm 2 (median, 8 years; range, 3 to 22 years), farm 3 (median, 10 years; range, 4 to 25 years), and farm 4 (median, 9 years; range, 2 to 23 years).

Association of reproductive status of mare with pregnancy loss, vaccination, and farm—Of the 480 mares of known reproductive status (barren, lost previous pregnancy, not bred, maiden, and foaling) that were vaccinated during the EE period, the incidence of EED did not differ significantly among reproductive statuses (barren, 1/27 [3.7%]; lost previous pregnancy, 3/34 [8.8%]; not bred, 0/5 [0%]; maiden, 1/69 [1.4%]; foaling, 19/326 [5.8%]). Of the 456 mares of known reproductive status that were vaccinated during the EF period, the incidence of EFL did not differ significantly among reproductive statuses (barren, 1/26 [3.8%]; slipped, 0/31 [0%]; not bred, 1/5 [20%]; maiden, 3/68 [4.4%]; foaling, 14/326 [4.3%]). Similarly, of the 437 mares of known reproductive status vaccinated during the LF period, the incidence of LFL did not significantly differ among reproductive statuses (barren, 1/25 [4.0%]; slipped, 1/31 [3.2%]; not bred, 0/4 [0%]; maiden, 2/65 [3.1%]; foaling, 18/312 [5.8%]). There was no significant difference in the incidence of LFL when comparing LFL among foaling mares with mares of other reproductive statuses.

Vaccination during the EE period differed significantly by reproductive status of mare: foaling mares were significantly ($P < 0.001$) less likely (106/345 [30.7%]) to have been vaccinated during the EE period than were mares of other reproductive statuses (68/135 [50.4%]). There was no significant difference in the proportion of mares vaccinated during the EF period by reproductive status of mare (whether considered as polytomous categories or by the dichotomous variable of foaling mares vs others). Foaling mares were significantly ($P < 0.001$) less likely to have been vaccinated during the LF period (53/311 [17.0%]) than were mares of other reproductive statuses (40/124 [32.2%]). Associations of vaccination during periods with foaling reproductive status appeared to be attributable to differences among farms. The proportions of foaling mares differed significantly ($P < 0.001$) among farms: the proportion of foaling mares at farms 1, 2, 3, and 4 were 62 of 62 (100%), 77 of 124 (62%), 103 of 157 (66%), and 103 of 137 (75%), respectively.

Multivariate modeling—Because vaccination was significantly associated with farm, multivariate models were fit to determine whether vaccination during a given period was significantly associated with pregnancy loss during that period after accounting for effects of farm. After adjusting for farm effects, there was no significant association of either EED or EFL with vaccination of pregnant mares during the EE period. After farm effects were accounted for, failure to have an EFL remained significantly ($P < 0.001$) associated with vaccination during the EF period: vaccinated horses were significantly less likely to have had an EFL, regardless of farm. After accounting for farm effects, there was no significant association of LFL with vaccination.

Discussion

In this study, the administration of killed WNV did not adversely affect pregnancy outcome. Administration of vaccine[a] during any of the 3 periods of pregnancy (ie, EE, EF, or LF period) was not associated with increased pregnancy loss during that same period or subsequent periods. The finding that mares vaccinated during the EF period were less likely to have an EFL may be a result of the relative length of this period (41 to 150 days of pregnancy) and the fact that vaccination during this period was common (breeding season begins in February, so many pregnancies are in the EF stage when vaccination for mosquito-borne diseases typically occurs), providing greater opportunity for vaccination during this period as mares remained pregnant. Use of methods for analysis of time-to-event data (eg, survival analysis) would likely have reduced the chances of a spurious protective effect of vaccination. Both the outcome of interest (pregnancy loss) and the primary exposure of interest (vaccination) were time-dependent and time varying, rendering analysis of temporality complex.

Although the sample size in this study was small and vaccination practices varied among farms, increased incidence of pregnancy loss was not associated with vaccination within or among farms. Variables that might have differed among farms (eg, timing of vaccination in relation to gestation period, age, and reproductive status) were considered to determine potential confounding. There was no association of reproductive status with pregnancy loss. However, there was a significant difference in timing of vaccination among mares with different reproductive status. The association of vaccination with reproductive status appeared to be attributed to differences among farms, which was largely attributable to a difference in numbers of foaling and nonfoaling mares included in the study. Nonfoaling mares are available for breeding earlier in the breeding season and should, typically, have more advanced pregnancies when vaccination for mosquito-borne diseases is performed. Additionally, farm 1 practiced a restricted, earlier-than-usual breeding program (beginning February 1), with > 80% of mares being foaling mares. Such farm variability in reproductive status of mares bred and date of onset of breeding activity likely explains the difference between farms in timing of vaccination by reproductive status of mare.

The overall incidence of pregnancy loss in mares included in this study was within the reported range of typical losses.[4,5] We did not evaluate whether vaccination prior to breeding would alter the frequency of pregnancy loss, but many practitioners take this approach, assuming potential adverse reactions (eg, fever) would be overcome by the time the mare is bred. However, because many mares are bred early in the year and thus are already pregnant at the onset of the mosquito season, our finding that vaccination of pregnant mares during the EE, EF, and LF periods was not associated with an increased incidence of pregnancy loss provides some confidence to the practitioner who must choose between vaccinating or not vaccinating a pregnant mare that may be exposed to the virus.

There were a number of limitations to this study in addition to those already mentioned. There is a certain amount of imprecision in the timing of when pregnancy losses occurred because mares were not checked for pregnancy status daily. This was part of the rationale for using analysis of time interval data. Losses were determined to have occurred between 2 successive pregnancy examinations, and intervals between successive pregnancy examinations typically increase after mares are determined to be pregnant at 45 days of gestation. This is due to a known reduction in risk of pregnancy loss as the pregnancy advances and failure for a mare to return to a fertile estrus once endometrial cups are formed (40 to 45 days) early in the fetal period, even if the pregnancy is terminated.[4] We do not believe this imprecision biased the results of our study.

The number of mares studied was relatively modest. Although this was not a safety study and there was no association of vaccination with pregnancy losses, a larger sample size would have had more statistical power to detect differences. However, the differences observed in the study reported here were small and not deemed clinically important. The extent to which our findings can be extrapolated to other populations is unknown, but the authors believe that these results would be applicable to other farms in North America.

Although assessing the impact of vaccination on passive transfer of immunity was not a primary aim of this study, we found no significant difference in the incidence of abortions or stillbirths (from the pregnancy produced in 2002) among mares vaccinated during the last 3 months of gestation, compared with pregnant mares not vaccinated during this period. This finding may indicate that this practice could be potentially safe for attempting to promote passive antibody protection through colostrums for suckling foals.

[a]Innovator, Fort Dodge Animal Health, Overland Park, Kan.
[b]S-PLUS 6.0, Insightful Inc, Seattle, Wash.

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