Temporality of early-term abortions associated with mare reproductive loss syndrome in horses

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Objective—To characterize the temporality of dates of breeding and abortion classified as mare reproductive loss syndrome (MRLS) among mares with abortions during early gestation.

Animals—2,314 mares confirmed pregnant at approximately 28 days after breeding from 36 farms in central Kentucky, including 515 mares that had early-term abortions.

Procedure—Farm veterinarians and managers were interviewed to obtain data for each mare that was known to be pregnant to determine pregnancy status, breeding date, last date known to be pregnant, and date of abortion.

Results—Mares bred prior to April 1, 2001, appeared to be at greatest risk of early-term abortion, both among and within individual farms. Mares bred in mid-February appeared to be at greatest risk of abortion, with an estimated weekly incidence rate of abortion of 66% (95% CI, 52% to 80%).

Conclusions and Clinical Relevance—Mares in central Kentucky bred between mid-February and early March were observed to be at greatest risk of early-term abortion, and risk gradually decreased to a background incidence of abortion of approximately 11%. Mares bred after April 1, 2001, appeared to be at markedly less risk, indicating that exposure to the cause of MRLS likely occurred prior to this date. (*Am J Vet Res* 2005;66:1792–1797)

During the spring of 2001, parts of north-central Kentucky were the sites of an apparent outbreak of early fetal losses, abortions, stillbirths, and neonatal foal deaths (collectively referred to as the **mare reproductive loss syndrome [MRLS]**); fibrinous pericarditis was also reported.[1–6] Although several epidemiologic studies of MRLS and related disorders have been reported,[1–6] the cause, pathogenesis, and many epidemiologic aspects of MRLS remain unknown.

The timing of adverse events during pregnancy can be helpful to indicate possible causes or characterize periods of particular vulnerability (eg, during organogenesis) for these events. To our knowledge, the temporality of MRLS with respect to date of occurrence (ie, month and day) and gestational age has not been reported. One report[6] indicated that the distribution of dates of onset of cases of fibrinous pericarditis was consistent with a common time-point exposure. A number of temporal questions and considerations exist for MRLS. The purpose of the study reported here was to characterize the temporality of dates of breeding and abortion among mares with abortions during early gestation classified as MRLS.

Materials and Methods

Study population—Veterinarians from 2 large equine clinics (Hagyard-Davidson-McGee Associates and Rood & Riddle Equine Hospital) in the Lexington, Ky, area were asked to provide a list of farms to which they provided equine reproductive services. For each farm, veterinarians from the 2 clinics provided rates of abortion for the farm (defined as the number of mares that were verified as pregnant at 28 days after breeding that subsequently lost the fetus at any stage of pregnancy divided by the number of mares verified as pregnant at 28 days after breeding) during 2000 and 2001. For purposes of this study, neonatal deaths were not considered in the definition of MRLS because most fetal losses were during the early fetal and late fetal periods[2–5] and because the data for fetal losses were readily available and accurate for categorizing farms. Farms in the upper 33rd percentile of abortion rate for 2001 or farms that had more than a 3-times increase in abortion rate during 2001 relative to 2000 were considered severely affected by MRLS and categorized as high-impact farms. Farms in the middle 33rd percentile that did not have a 3-times increase in abortion rate during 2001 relative to 2000 were considered moderately affected by MRLS and were categorized as moderate-impact farms. Farms in the lower 33rd percentile of abortion rate for 2001 were categorized as low-impact farms. Categorization of farms was performed prior to obtaining any temporal data used in this study.

Data collection and statistical analyses—Farm managers or owners were contacted to solicit participation; those that did not respond to 3 attempts to reach them by telephone and by letter were considered nonrespondents. For each farm, the farm's veterinarian or manager was asked to provide the following data for each mare at the farm that was bred at the farm and determined to be pregnant at approximately 28 days of gestation: name or farm identification number of the mare; last breeding date (hereafter referred to

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as breeding date); whether the mare had **early-term abortion (ETA)**; if ETA occurred, age at which the mare was last determined to be pregnant; and date at which mare was determined to have had an abortion. An ETA was defined as abortion (ie, absence of a viable fetus) at ≤ 90 days of gestation in a mare that was determined to be pregnant at approximately 28 days of gestation. The longitude and latitude were determined for each farm included in the study.

Data analysis relied on graphical methods and formal statistical inference for key hypotheses. The frequency distribution of breeding dates was plotted as a function of time by use of histograms and smoothed density plots with a Gaussian window.[7] Density plots with a Gaussian window are density estimates constructed by weighted averaging of event counts in overlapping windows (in this case, windows of calendar time), with the weights determined by use of the Gaussian (ie, standardized bell–shaped) density function. Density plots were created for all mares and for mares by status (abortion vs no abortion) and were stratified by farm MRLS status (high, moderate, or low impact). Density plots were scaled so that the maximum value was always 1, to facilitate comparison of distributions among the 3 categories of farms. The χ^2 test was used to compare the proportion of mares bred prior to March 1, 2001, among the 3 categories of MRLS status.[8] The Wilcoxon rank sum test was used to test the null hypothesis of equivalent distributions for mares with and without abortion in each of the 3 categories of farms[8]; $P < 0.05$ was used to determine significance for this analysis. On the basis of the dichotomous outcome of abortion, the probability (incidence rate) of abortion for mares was modeled as a function of calendar time by use of **generalized estimating equations (GEEs)**[9] to account for farm-level clustering among mares; a 1-week interval was used for statistical modeling. The relative risk of abortion for mares bred before and after April 1, 2001, was assessed by use of GEE. Ninety-five percent **confidence intervals (CIs)** were calculated for week-specific abortion incidences by use of robust estimators.[9] For mares in the sample for which the last date bred, last date known pregnant, and abortion status were known, event-time graphics were used to present data, ordered from top to bottom of the figure by breeding date. To assess spatio-temporal distribution of breeding dates, the longitude and latitude of each participating farm were determined. These data were used to create 16 overlapping subregions of the sampling frame of farms; overlapping subregions were used to diminish discontinuities (ie, to avoid subregions with little or no data) that interfered with interpretation of spatial processes. Plots took the form of a north-to-south gradient from the direction of top to bottom of the page and an east-to-west direction from right to left of the page. Breeding dates for nonaborting and aborting mares were plotted for each of the 16 subregions to provide a graphical basis for interpreting differences in distribution of breeding dates by region.

Results

Data were provided for 2,314 mares from 36 farms. Abortion was reported for 515 (22%) mares. Of these 515 mares, the putative date of abortion was provided for 468 (91%) mares and the last date known pregnant was provided for 313 (61%) mares. Seventeen farms were classified as high impact, 5 farms were classified as moderate impact, and 14 farms were classified as low impact. Distribution of breeding dates for mares included in the study revealed considerable overlap among the 3 categories of MRLS status of farms; however, mares at the high-impact farms appeared to have more concentrated breeding dates in mid-February (**Figure 1**). The proportion of mares bred prior to March 1, 2001, differed significantly ($P < 0.001$) among the high-impact (177/942 [19%]), moderate-impact (88/719 [12%]), and low-impact (74/621 [12%]) farms. Scaled density plots (**Figure 2**) of breeding dates stratified by abortion status and MRLS impact status revealed a number of patterns. First, the breeding dates for mares that aborted appeared to be earlier than for mares that did not abort, with a fairly sharp rise in frequency of breeding around February 15, 2001. Second, this pattern was most apparent for the high- and moderate-impact farms. Third, the distribution of breeding dates for mares that did not abort appeared similar, regardless of the MRLS-impact status of the mares' farms. Fourth, the distribution of breeding dates of mares that aborted at low-impact farms appeared to be biphasic. Wilcoxon tests of common distributions of breeding dates for both categories of mares all rejected the null hypothesis of a common distribution.

On the basis of all available data, a GEE was used to test the null hypothesis of common risk of abortion for mares bred before April 1, 2001, compared with mares bred on or after that date, accounting for clustering of mares within farms. The relative risk was estimated at 0.163 (95% CI, 0.107 to 0.250; $P < 0.001$), indicating a strongly protective effect for later breeding.

The week-specific estimates of abortion incidence rates among successful breedings (hereafter referred to as breedings) determined with the GEE regression model were plotted as a function of calendar time (**Figure 3**). The background weekly incidence rate of abortion, estimated by use of the data from study mares bred after May 30, 2001, was 11.4 abortions/100 breedings (95% CI, 6.0 to 16.8 abortions/100 breedings).

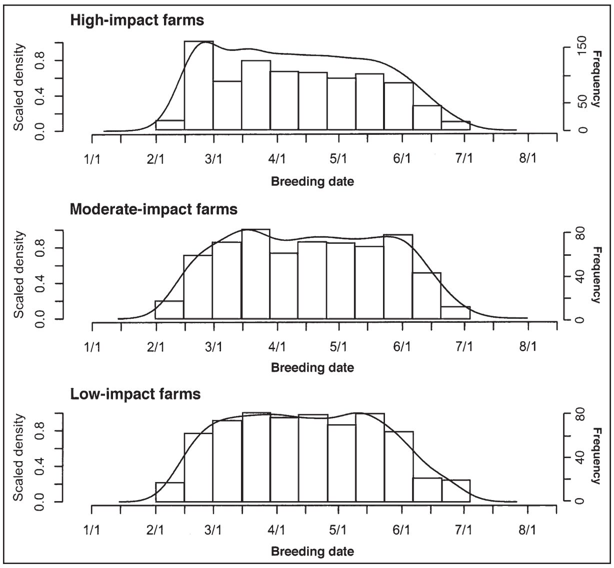

Figure 1—Distribution of breeding dates for 2,314 mares that were confirmed pregnant at approximately 28 days after breeding from 36 farms in central Kentucky during 2001, including 515 mares that had early-term abortions (ETAs), stratified by level of mare reproductive loss syndrome (MRLS) impact.

The abortion incidence rate was significantly greater than the background rate for breeding dates from day 37 to day 86 (February 6, 2001, to March 27, 2001). The peak of the estimated incidence rate was approximately 66 abortions/100 breedings (95% CI, 52 to 80 abortions/100 breedings).

Event-time data (date last known pregnant or putative date of abortion) were only reported for 1,617 mares. The event-time graphics indicated that mares bred up until the date of March 28, 2001, had the highest frequency of early fetal loss (**Figure 4**).

Evaluation of spatiotemporal graphics indicated that there was less breeding activity in the northwest and southeastern segments, compared with other segments (**Figure 5**). The distributions of breeding dates of mares that aborted appeared similar among those regions in which breeding activity was more typical (ie, regions other than those in the northwest and southeast, where breeding was less frequent; **Figure 6**). There was no apparent pattern of migration of the breeding date of greatest risk with respect to space (ie, across the regions sampled); the breed-

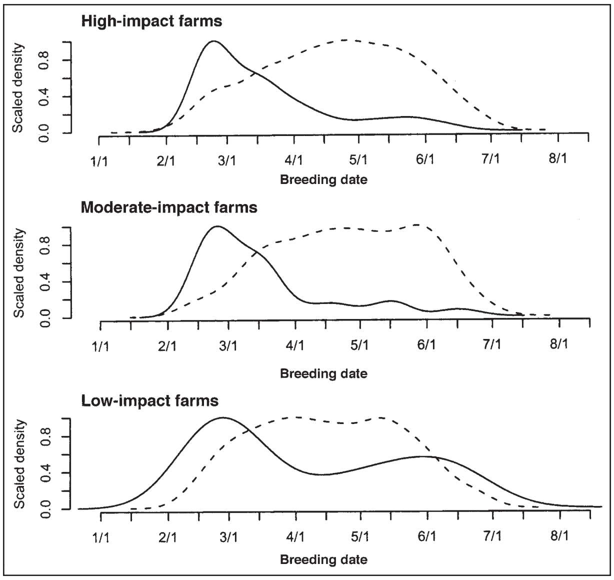

Figure 2—Scaled density plots of breeding dates for 2,314 mares that were confirmed pregnant at approximately 28 days after breeding from 36 farms in central Kentucky during 2001, including 515 mares that had ETAs, stratified by abortion status and MRLS impact status. Dashed lines represent the breeding dates of nonaborting mares, and solid lines represent the breeding dates of mares that had an ETA.

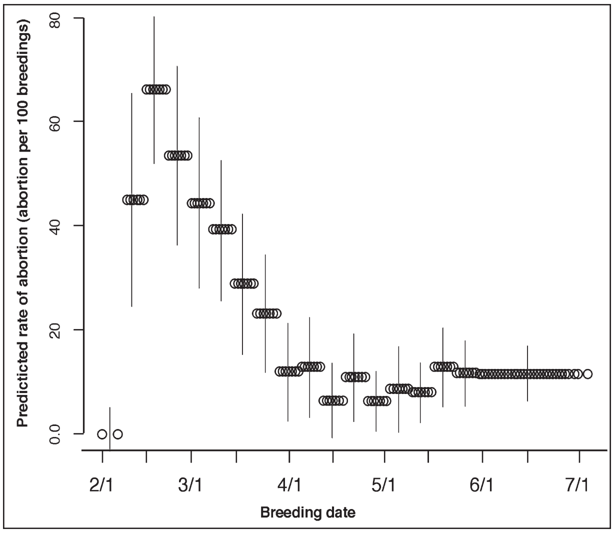

Figure 3—Week-specific estimates of early-term abortion incidence rates, as determined by use of the generalized estimating equations regression model, as a function of calendar time among 2,314 mares that were confirmed pregnant at approximately 28 days after breeding from 36 farms in central Kentucky during 2001. Bars represent the 95% confidence interval for the estimated weekly incidence rate; circles represent breeding dates for which data from at least 1 mare were available.

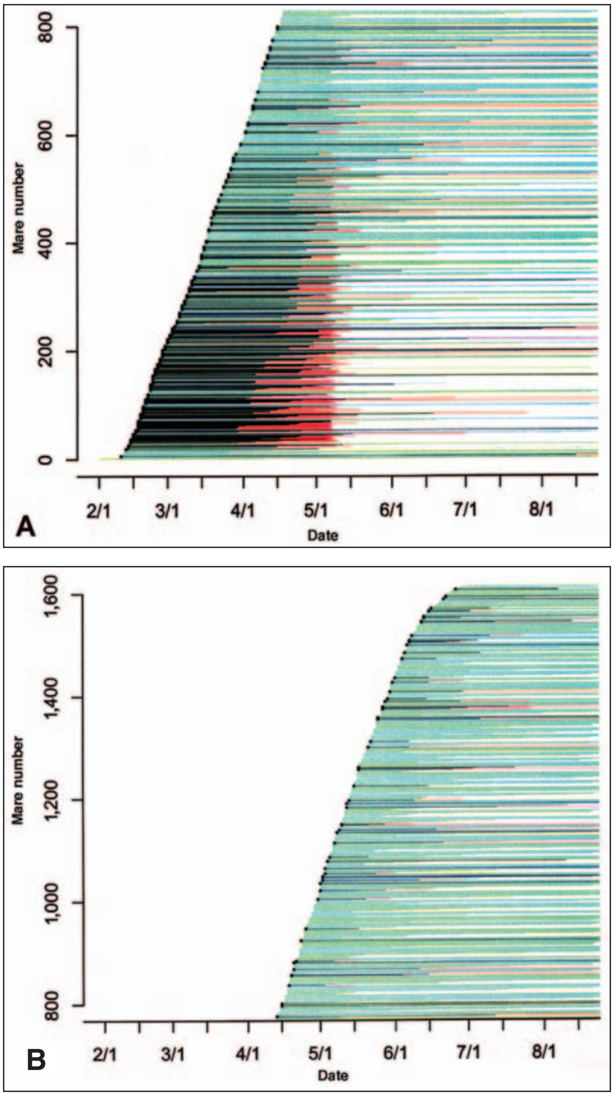

Figure 4—Event-time intervals for 1,617 mares bred in Kentucky between February and April 2001. Color is used to encode the state of each mare across reported dates of events. Each mare is represented by a horizontal line with segments of different colors: green represents the time from last breeding date to last date known pregnant; black represents the time from last breeding date to last date known pregnant for a mare that aborts; and red represents the time from last date known pregnant to date that loss of pregnancy was determined. Length of line segments representing time from last date known pregnant to estimated date of abortion indicates the uncertainty or imprecision of each of these estimates as an indicator of the date of fetal loss or abortion. A—Mares 1 to 800. B—Mares 801 to 1,617.

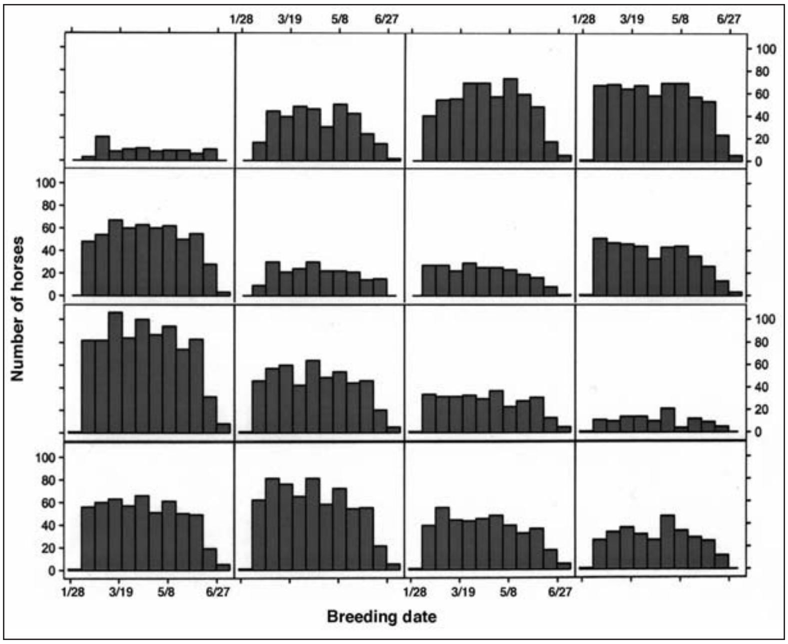

Figure 5—Trellis plots of breeding date densities of 1,799 nonaborting mares from 36 farms in Kentucky during 2001, within overlapping subsets of data formed by dividing the distribution of farm longitude and latitude into 4 segments. The vertical axis for each trellis segment is the count (ie, number of mares), and the horizontal axis of each trellis segment represents calendar date.

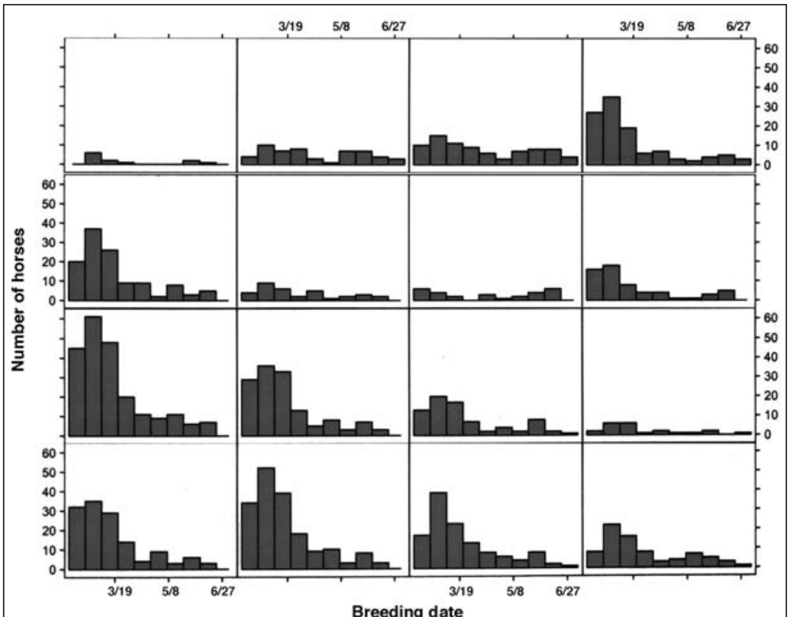

Figure 6—Trellis plots of breeding dates among 515 mares from 36 farms in Kentucky that aborted during 2001, within overlapping subsets of data formed by dividing the distribution of farm longitude and latitude into 4 segments. The vertical axis for each trellis segment is the count (ie, number of mares), and the horizontal axis of each trellis segment represents calendar date.

ing dates of mares that aborted appeared similar for all regions.

Discussion

Results of this study indicated that mares bred earlier during 2001 were at greatest risk of ETAs, compared with mares bred later. The distributions of breeding dates of all mares that became pregnant appeared similar among farms irrespective of their MRLS-impact category, but the breeding dates of mares with ETAs appeared to be earlier than those of mares that did not abort. This finding could be observed both collectively and at the farm level for mares from farms categorized as moderate and high impact regarding MRLS. The distinction between breeding dates for mares that did and did not abort from the same farm was most apparent early in the year (prior to March 15, 2001); the median last breeding date was before April 1 for all but 3 of the high-impact farms and for all of the moderate-impact farms. In contrast, the boxplots of data from low-impact farms revealed little separation of breeding dates between aborting and nonaborting mares, with 2 exceptions. It is possible that the latter 2 farms were MRLS-affected farms. In the absence of pathognomonic lesions or specific diagnostic criteria for ETAs caused by MRLS, classification of farms with respect to impact of MRLS was made on the basis of incidence proportions of abortion, without consideration of cause of abortion. Thus, abortions at some high-impact farms might have been attributable to causes other than MRLS, and some (if not many) abortions at low-impact farms might have been attributable to MRLS. It is noteworthy that the distribution of breeding dates for mares at low-impact farms included a peak in late February 2001, similar to that seen at the moderate- and high-impact farms.

The association of last breeding date with the weekly incidence rate of abortion appeared to reach a sharp peak in mid-February 2001, with a magnitude of approximately 66 abortions/100 breedings. The incidence appeared to decrease smoothly until the beginning of April to an apparent background incidence of approximately 11 abortions/100 breedings. The distribution of breeding dates did not provide clear evidence regarding causation or character of the MRLS epidemic. At least 2 hypotheses can be posed on the basis of the findings of this study. The observed distribution of incidence by breeding dates could be explained by a point-source epidemic in which a pathogen or toxin emerged and rapidly departed in a focused calendar period with severe impact on pregnancies during that interval and a gradual tapering off of effect on later pregnancies. The observed distribution also could be explained by a chronic-source epidemic in which a pathogen emerged and then decreased during a protracted calendar period, with the impact on abortion incidence proportion-al to the presence of the pathogen or toxin. Examining the distribution of dates of abortion would have added valuable information to differentiate between these 2 hypotheses and otherwise characterize the temporality of the epidemic, but these data were not available. Knowledge of the apparent background rate of abortion for mares at these farms may be valuable for purposes of future surveillance and monitoring.

The event-time figures with graphical representation of breeding date, date last known pregnant, and date determined to no longer be pregnant indicated that abortions that appeared to occur in late April to early May were generally associated with breeding dates prior to mid-March and that abortions in general and in late April to early May in particular were rare for breedings subsequent to April 1, 2001. Because there is no single etiology of ETAs, the fact that they were observed after breeding dates late in the season was not unexpected. Interpretation of these data must be made with caution because of the uncertainty about the precise time at which abortion occurred because data were only available for time at which mares were detected to no longer be pregnant (for most mares that aborted) and date at which the mare was last known to be pregnant (for only about 60% of mares). Veterinarians and farm personnel monitored pregnancy status closely during the first 90 days of gestation, but they were not monitored daily; therefore, a precise date of abortion was indeterminable for all mares.

Temporospatial analysis failed to reveal any evidence of a pattern (migration) or other inhomogeneity across geographic regions of the breeding dates of mares that aborted. Because farms studied were not selected randomly and because the data from some regions were less than from others, there is great need for a more rigorous temporospatial analysis of the MRLS epidemic.

This study used a variety of graphical methods to represent the temporality of breeding dates and MRLS-associated pregnancy losses of mares. Because we did not know the distribution of abortion dates, it was not possible to construct an epidemic curve by plotting the frequency of abortion by calendar date (or breeding date). Moreover, a general epidemic curve would have failed to reveal farm-specific effects; some of the graphical methods used (eg, boxplots) were considered more space-efficient than plotting panels of farm-specific epidemic curves. Other authors have used survival methods to make inferences about the cause and temporality of late-term abortions attributed to MRLS[9]; because the date of pregnancy loss for ETAs (early fetal losses) was generally not known and because many of the results pertained to breeding-date data (ie, not time-to-event data), such methods could not be applied accurately to the data reported here.

There were a number of other limitations to this study. Ideally, a systematic probability sampling of all mares in the counties in and around Lexington, Ky, would have been selected for inclusion in the study. Because a listing of all mares was not available, we obtained data from veterinarians in 2 large practices in this area who were willing to share data from mares at all farms they serviced. Although the regional geographic distribution and population sizes of farms participating in the study (range, 10 to 399 mares) were diverse, we did not know the proportion of farms in the study area for which these practices provided services. Although a complete farm census for the region was not available to us, anecdotally we understand that the 2 participating practices provide services to a large proportion of farms in the study area. Thus, it is unclear to what extent our results can be extrapolated beyond this reference population.

Because there are no specific criteria for diagnosing ETAs or other abortions associated with the cause of MRLS, it is impossible to determine which of the ETAs included in this study were MRLS associated and which were not. The MRLS epidemic was defined as an excessive rate of abortions among mares in central Kentucky during 2001. We used the distribution of abortion rates during 2001 and the magnitude of abortion in 2001 relative to 2000 to define farms with respect to apparent impact of MRLS. In the absence of pathognomonic clinical and pathologic findings or specific, accurate methods for diagnosing MRLS, a certain amount of imprecision will exist in attributing abortion in a given mare to MRLS.

The temporality of ETAs associated with MRLS could have been best investigated by knowing the exact dates of conception (fertilization of the ovum) and of abortion. In this study, the last recorded breeding date was used as an indicator of the time of conception, thereby introducing a small amount of uncertainty in estimates of duration of pregnancy. Considerably more imprecision resulted from lack of knowledge of the specific time at which fetal loss occurred.

The principal aim of this study was to clarify the temporality of MRLS abortions. Because the study was conducted retrospectively, there were limitations with respect to the quality of the data pertaining to the principal outcome of interest, abortion. In particular, the time of fetal loss or abortion could not be determined with any certainty. For most mares, the date at which the mare was identified as being no longer pregnant was reported; however, because mares were not serially monitored on a frequent basis, the date at which the mare was detected to be no longer pregnant could not be considered to be an accurate representation of the precise or approximate date of abortion. The age at which the mare was last known to be pregnant was provided for only a little more than half of the mares that aborted. As represented in the event-time figures, the interval from last date known to be pregnant until date known to be not pregnant was often long. Thus, we were unable to assess issues pertaining to the temporality of the outbreak with respect to gestational age (eg, ages at which the fetus was particularly susceptible or resistant to MRLS).

Results of this study provide evidence that ETAs occurred most often among mares bred between mid-February and late March and that the mares bred in mid-February were at greatest risk. It also appeared that the relationship between breeding date, duration of gestation, and incidence of ETA was complex (ie, there were no simple relationships between ETAs and breeding date and gestational age, such as a specific gestational age at which fetuses were not susceptible or a particular breeding date after which ETAs did not occur). Studies with more detailed information about the precise gestational age of abortion and with a method for confirming the diagnosis of MRLS-associated fetal losses are needed to further clarify the temporality of MRLS-associated fetal losses.

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