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Case-control study of early-term abortions (early fetal losses) associated with mare reproductive loss syndrome in central Kentucky

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Objective—To identify factors associated with abortions during early gestation classified as mare reproductive loss syndrome (MRLS).

Design—Case-control study.

Animals—324 broodmares from 43 farms in central Kentucky, including 121 mares from 25 farms that had early-term abortions (ETAs) associated with MRLS (case horses), 120 mares from the same farms but that did not abort, and 83 mares from 18 farms that were not severely impacted by MRLS.

Procedure—Farm managers were interviewed to obtain data on various management practices and environmental exposures for the mares. Data for case and control horses were compared to identify risk factors for mares having MRLS-associated ETAs.

Results—Several factors increased the risk of MRLS-associated ETAs, including feeding hay in pasture, greater than usual amounts of white clover in pastures, more eastern tent caterpillars in pastures, abortion during a previous pregnancy, and sighting deer or elk on the premises.

Conclusions and Clinical Relevance—Analysis indicates that certain characteristics of pastures predisposed mares to MRLS-associated ETAs. Methods for limiting exposure to pasture (keeping mares in stalls longer) during environmental conditions similar to those of 2001 (ie, sudden freezing in mid-April following warmer-than-usual springtime temperatures and larger-than-usual numbers of eastern tent caterpillars in and around pastures) should reduce the risk of mares having MRLS-associated ETAs. It was not possible to determine whether exposure to white clover or caterpillars were causal factors for MRLS or were merely indicators of unusual environmental conditions that resulted in exposure of mares to a toxic or infectious agent. (*J Am Vet Med Assoc* 2003;222:210–217)

During the spring of 2001, an epidemic of abortions and stillbirths occurred in central Kentucky. This epidemic of abortions was identified as **mare reproductive loss syndrome (MRLS)**.[1] Although the magnitude of morbidity and mortality of the epidemic has not been completely described, the experience of 2 of the authors (SEB, TWR) indicated that many of these abortions were in early gestation (ie, the first 90 days of gestation). The cause of MRLS remains unknown. Identification of factors predisposing mares to having MRLS would provide clues of potential causal factors and a basis for developing preventive strategies. The objective of the study reported here was to describe results of a case-control study in which mares that had MRLS-associated **early-term abortions** (**ETAs**; early fetal losses) were compared with 2 groups of control mares to identify factors that could predispose mares to having MRLS-associated ETAs.

Materials and Methods

Study population—Veterinarians from 2 large equine clinics[a,b] in Lexington, Kentucky, were asked to provide a list of farms to which they provided equine reproductive services. Horses that received care from veterinarians from these 2 clinics were the reference population for a study[2] of MRLS-associated late-term abortions; although there was some overlap, the study populations and methods for the late-term abortion study and the study reported here were distinct. For each farm, veterinarians from the 2 clinics also provided rates of pregnancy losses for the farm (defined as the number of mares that were verified as pregnant at 28 days after breeding that subsequently lost the fetus divided by the number of mares verified as pregnant at 28 days after breeding) during 2000 and 2001. The distribution of loss rates for farms was examined, and farms were characterized as high-impact or low-impact. Farms in the upper 33rd percentile of pregnancy loss rate for 2001 or farms that had more than a 3-fold increase in pregnancy loss rate during 2001 relative to 2000 were considered severely affected by MRLS and categorized as high-impact farms. Farms in the lower 33rd percentile of pregnancy losses for 2001 were categorized as low-impact farms. Categorization of farms was made prior to obtaining any data regarding predictors of outcome. At each high-impact farm, 5 mares that had ETAs during the period between February 1 and July 1, 2001, were randomly selected as ETA cases (ETA group). Farm managers were instructed to identify mares that aborted during the first 90 days of pregnancy and that were bred during the period from February to April 2001. When fewer than 5 such mares were available, mares bred during February, March, or April that aborted fetus-

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es > 90 days of age or mares that were bred after April were included. Two groups of control mares were identified. The first control population (FARM group) comprised mares from the same farm (5 mares/farm) as the case mares but that did not abort and that were matched on the basis of breeding date (± 14 days) with the case mares. The second control population (LOW-IMPACT group) was obtained from low-impact farms. At each low-impact farm, 5 mares that were bred and did not abort during 2001 were matched on the basis of breeding date (± 14 days) with case mares for inclusion in the LOW-IMPACT group. For case and control farms, when fewer than 5 mares were available for any group, all available mares were included.

Data collection and analysis—Farm managers or owners were contacted to solicit participation; those who did not respond to 3 attempts to reach them by telephone and by letter were considered nonrespondents. For each case or control horse at participating farms, information about the following general categories was recorded on a questionnaire[c]: name or farm identification number of the mare, signalment, breeding date and reproductive history (including estimated date of fetal loss or abortion), duration during which the mare resided in Kentucky, feeding practices, watering practices, stabling or housing practices, pasture characteristics, pasture management practices, vaccination and deworming history, exposure to wildlife, and exposure to eastern tent caterpillars. Date of fetal loss was used as an index date for comparing events during the 4-week period prior to this index date of abortion for case and control mares.

The association between categoric variables (eg, breed and ETA status) was summarized by use of contingency tables for categoric data and medians and ranges for continuous data. These summaries neglected the correlation among observations from the same farm (ie, multiple case mares submitted from the same farm and the same farm contributing to the ETA group and FARM or LOW-IMPACT groups) and were used strictly for descriptive purposes.

To account for correlations among mares from the same farm when examining the association between a given covariate and ETA for the comparison of the ETA group and LOW-IMPACT group, an extension of generalized linear modeling referred to as generalized estimating equations was used for analysis.[3] Modeling stipulated a dichotomous outcome (ETA group or LOW-IMPACT group). Coefficients of the models had the interpretation of being the natural logarithm of the **odds ratio (OR)** of being in the ETA group for the individual dependent variables evaluated. Robust standard errors were used to calculate 95% confidence intervals, and robust Z values were used to assess statistical significance.[3] Each independent variable was examined separately for association with ETA. Variables associated with ETA at a value of $P < 0.10$ were included in multivariate modeling. A forward stepwise approach was used to select variables included in the multivariate model on the basis of the magnitude of the observed association. Data were analyzed by use of commercial software.[d]

Comparison of the ETA group and FARM group was made by use of conditional logistic regression[4] to identify factors associated with a mare being in the ETA group. Each independent variable (eg, age) was examined separately for association with ETA, and the association between a given exposure (eg, being in pasture after midnight) and disease was expressed as the OR, derived from analyses by use of conditional logistic regression. Variables associated with ETA at a value of $P < 0.10$ were included in multivariate modeling. A forward stepwise approach was used to select variables included in the multivariate model on the basis of magnitude of the observed association.[4] Data were analyzed by use of commercial software.[d]

Results

Study population—Information was provided for 45 farms by 1 of the large equine clinics and for 16 farms by the other large equine clinic. The upper 33rd percentile of fetal losses among these 61 farms ranged from 25 to 63% (n = 20 farms), whereas the range for the lower 33rd percentile was 2 to 13% (20), and the middle 33rd percentile ranged from 14 to 24% (21). There were 7 farms in the middle 33rd percentile that had a ratio of pregnancy loss rates in 2001 to 2000 that was > 3 (median, 4.1; range, 3.1 to 6.6). Pregnancy loss rates for these 7 farms during 2000 ranged from 3 to 6% (median, 5%), whereas during 2001 they ranged from 17 to 23% (median, 21%). Thus, there were 47 farms eligible for inclusion in the study. Of these 47 farms, 43 (91.5%) provided data. Of the 4 farms that did not provide data, 1 was sold such that historical data could not be obtained, 1 declined to participate, and 2 did not respond to repeated attempts to schedule interviews for data collection.

The date on which each mare was examined and it was determined that the mare was no longer pregnant was determined for mares in the ETA group; dates ranged from March 19 to December 21, 2001. Age of the fetus and last date at which it was verified that the fetus was alive were recorded for mares in the ETA group; fetal age at last determination of viability ranged from 16 to 205 days (median, 60 days). Viability of 3 fetuses was last determined when fetuses were < 30 days of age (16, 21, and 29 days, respectively). Viability of only 3 fetuses was last determined when fetuses were > 90 days of age (120, 202, and 205 days, respectively). Thus, 97.5% of fetuses had the last determination of fetal viability when they were ≤ 90 days of age.

Comparison of ETA group with LOW-IMPACT group—Data were compared between the ETA group and LOW-IMPACT group.

Farms and residency of mares

The ETA group comprised 121 mares from 25 farms (median, 5 mares/farm; range, 3 to 6 mares/farm). Two farms provided all 6 mares affected at the farm. The LOW-IMPACT group comprised 83 mares from 18 farms (median, 5 mares/farm; range, 2 to 5 mares/farm). For the ETA group, 18 farms were categorized as high impact (ie, upper 33rd percentile of the distribution of pregnancy loss rates), and 7 were categorized as moderately affected with a ratio of > 3 for pregnancy loss rates for 2001 relative to 2000. Pregnancy loss rates during 2001 for the ETA group ranged from 17 to 63% (median, 27%), whereas pregnancy loss rates for the LOW-IMPACT group ranged from 2 to 13% (median, 9%).

Although the number of mares at each farm of the ETA group (median, 45 mares; range, 6 to 292 mares) was greater than that of the LOW-IMPACT group (median, 31 mares; range, 2 to 120 mares), these values did not differ significantly. We did not detect significant differences between the ETA and LOW-IMPACT groups with regard to number of horses sharing the pasture, total amount of land (hectares) for each farm, total amount of pasture in which mares were maintained, duration during which the mare had resided in Kentucky, or duration of residence of the mare at the current farm.

Age, parity, and breed

Age of mares in the ETA group (median, 8 years; range, 3 to 19 years) was similar to that for mares in the LOW-IMPACT group (median, 8 years; range, 2 to 20

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years). Parity of mares was similar for the ETA (median, 2 foals; range, 0 [nulliparous] to 11 foals) and LOW-IMPACT (median, 2 foals; range, 0 to 12 foals) groups. All mares in the ETA group were Thoroughbreds, whereas 76 (91.6%) mares of the LOW-IMPACT group were Thoroughbreds. Other mares in the LOW-IMPACT group were 2 Quarter Horse mares from 1 farm and 5 mares described as other (3 Tennessee Walking Horses from 1 farm, and 1 Paint and 1 Warmblood-Thoroughbred crossbred from another farm).

Breeding history

The last breeding date for the ETA group ranged from February 10 to June 4, 2001 (median, February 28). The last breeding date for the LOW-IMPACT group ranged from February 6 to June 2, 2001 (median, March 15). The percentage of mares at the farm that were bred before April 1, 2001, for the ETA group (median, 39%; range, 7.5 to 77%) was similar to that of the LOW-IMPACT group (median, 39%; range, 0 to 70%). All but 1 of the mares in the ETA group and 2 mares in the LOW-IMPACT group were bred in Kentucky. Mares in the ETA group were significantly ($P = 0.022$) more likely than mares in the LOW-IMPACT group to have had a previous abortion during the 5-year period prior to 2001 **(Table 1)**. There was not a significant difference between the 2 groups in the number of mares that were transported to the farm for breeding.

MRLS

For all 121 mares in the ETA group, it was reported that there were other mares or foals on the farm that were affected by MRLS-associated conditions, whereas there were only 70 (84%) mares in the LOW-IMPACT group for which it was reported that there were other mares or foals on the farm that were affected by MRLS-associated conditions. Although a history of late-term abortions associated with MRLS at the farm was more common for the ETA group (75/121, 62%) than for the LOW-IMPACT group (37/83, 45%), percentages for the 2 groups did not differ significantly. A history of pericarditis was significantly ($P = 0.040$) greater among the ETA group than the LOW-IMPACT group (Table 1). All 10 mares with a history of uveitis attributed to MRLS were from the ETA group.

Feeding practices

The ETA and LOW-IMPACT groups did not differ significantly with respect to the proportion of mares fed any type of concentrate, amount of concentrate fed daily, proportion of mares fed concentrate in pasture, proportion of mares fed concentrate on the ground in pasture, number of times mares were fed daily, or proportion of mares with a history of a recent change in diet.

Most horses in the ETA (77/121, 64%) and LOW-IMPACT (47/78, 60%) groups were fed a combination of grass and alfalfa hay during the 4-week period prior to the index abortion; there was not a significant difference between the groups with respect to the type of hay fed during this period. All mares in the ETA and LOW-IMPACT groups were fed hay harvested during 2000. There was no significant difference between the 2 groups in the proportion of mares fed homegrown hay or fed hay grown elsewhere in Kentucky. Whether hay was fed exclusively in stalls (rather than exclusively at pasture or partially in a stall and partially at pasture) was significantly ($P = 0.005$) associated with ETA (Table 1). For the ETA group, only 10 (8%) mares were fed hay exclusively in a stall, compared with 43 (52%) mares for the LOW-IMPACT group. The proportion of mares fed exclusively at pasture was similar for both groups (ETA group, 8%; LOW-IMPACT group, 12%), but there were many more mares in the ETA group fed hay partially in a stall and partially at pasture (101/121, 83%), compared with the proportion of mares in the LOW-IMPACT group (30/83, 36%).

Table 1—Comparison of characteristics of 121 mares with early-term abortions (ETAs) associated with the mare reproductive loss syndrome (MRLS; ETA group) and 83 mares from farms least affected by MRLS but that were matched (± 14 days) on the basis of breeding date with mares in the ETA group (LOW-IMPACT group)

Variable	ETA group	LOW-IMPACT group	OR (95% CI)*	*P* value
Abortion during preceding 5 years				
Yes	26 (22%)	8 (10%)	2.7 (1.1, 6.4)	0.026
No	90 (78%)	74 (90%)	1	NA
Mares with MRLS-associated pericarditis at farm				
Yes	25 (21%)	2 (2%)	10.5 (1.1, 99.5)	0.040
No	96 (79%)	81 (98%)	1	NA
Hay fed in pasture during 4-week period prior to index abortion				
Yes	111 (92%)	40 (48%)	11.9 (2.1, 67.0)	0.005
No	10 (8%)	43 (52%)	1	NA
Rapid growth of some plant life in pasture in an amount that was larger than expected during the 4-week period prior to index abortion				
Yes	96 (83%)	37 (47%)	5.3 (1.2, 22.6)	0.024
No	20 (17%)	41 (53%)	1	NA
Rapid growth of white clover in pasture in an amount that was larger than expected during the 4-week period prior to index abortion				
Yes	91 (78%)	27 (35%)	6.9 (1.7, 28.4)	0.008
No	25 (22%)	51 (65%)	1	NA
Excess of white clover in pasture during April and May 2001 relative to April and May 2000				
Yes	111 (96%)	50 (64%)	12.4 (1.3, 117.2)	0.028
No	5 (4%)	28 (36%)	1	NA
Elk or deer seen at premises during preceding 12 months				
Yes	46 (40%)	8 (10%)	5.8 (> 1.0, 32.3)	0.047
No	70 (60%)	70 (90%)	1	NA
Heavy burden of caterpillars during 2001 (relative to moderate or low burden)				
Yes	106 (91%)	52 (63%)	6.3 (1.1, 36.0)	0.038
No	10 (9%)	31 (37%)	1	NA
Caterpillars observed in all (100%) pastures during 2001				
Yes	72 (60%)	15 (18%)	6.7 (1.5, 29.3)	0.012
No	49 (40%)	68 (82%)	1	NA

*Odds ratios (OR) were derived by use of generalized estimating equations. 95% CI = 95% confidence interval. NA = Not applicable.

The ETA and LOW-IMPACT groups did not differ significantly in the proportions that were fed a supplement during the 4-week period prior to the index abortion, were fed a calcium supplement during pregnancy, had access to a mineralized salt block, were fed loose mineralized salts, had access to a regular (ie, sodium chloride) salt block, or had access to loose regular salt.

Watering practices

The ETA and LOW-IMPACT groups did not differ significantly with respect to source of water (municipal,

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well, or pond), water containers or vessels (bucket, trough, automatic waterer, or other type of vessel), or frequency with which water containers were cleaned.

Stabling or housing practices

All mares in the ETA and LOW-IMPACT groups had access to pasture during the 4-week period prior to the index abortion. The ETA and LOW-IMPACT groups did not differ significantly in the number of hours per day that a mare spent in a stall or a paddock during each week for the 4-week period prior to the index abortion, proportion of mares that had access to pasture after midnight, or estimated proportion of the mare's daily dietary intake derived from pasture during the 4-week period prior to the index abortion.

Pasture characteristics

Although mares in the ETA group (32/121, 26%) were less likely than mares in the LOW-IMPACT group (45/83, 54%) to have access to pasture that was grass only (as compared to grass and legumes), these percentages did not differ significantly. The predominant type of grass in pastures of mares in both groups was bluegrass alone (29/121 [24%] for ETA group; 25/78 [32%] for LOW-IMPACT group), bluegrass in combination with orchard grass and white clover (53/121 [44%] for ETA group; 33/78 [42%] for LOW-IMPACT group), or orchard grass and fescue (34/121 [28%] for ETA group; 5/78 [6%] for LOW-IMPACT group); these values also did not differ significantly.

Growth that was considered larger than usual for some plant life during the 4-week period prior to the index abortion case was significantly ($P = 0.024$) more likely for pastures grazed by mares in the ETA group than for pastures grazed by mares in the LOW-IMPACT group (Table 1). This difference appeared to be attributable to a significant ($P = 0.008$) difference in the amount of white clover that was considered larger than usual in the pastures of mares in the ETA group relative to mares in the LOW-IMPACT group. Although most mares in both groups had an excess of white clover in pastures during April and May 2001 relative to April and May 2000, this finding was significantly ($P = 0.028$) more common for mares in the ETA group than for mares in the LOW-IMPACT group.

The ETA and LOW-IMPACT groups did not differ significantly with respect to a history of hemlock observed in pasture during the 4-week period prior to the index abortion, history of fescue toxicosis at the farm, history of pastures described as being lush during the 4-week period prior to the index abortion, whether the pasture in which the mare resided during the 4-week period prior to the index abortion had been used before as pasture, and whether the pasture in which the mare resided during the 4-week period prior to the index abortion had been rested from grazing by horses. There were not any significant differences between the 2 groups regarding whether the pasture had been fertilized during the 4-week period prior to the index case of abortion, whether the pasture in which the mare resided had been fertilized during the fall of 2000, whether lime had been applied during either 2000 or 2001 to the pasture in which the mare resided, whether pastures had been mowed during the 4-week period prior to the index abortion, whether pastures had been mowed before the frost in mid-April of 2001, number of times the pasture was mowed between January 2001 and May 2001, and frequency of manure being spread on the pasture in which the mare resided. None of the grazed pastures had herbicides applied during the 4-week period prior to the index abortion. Only 3 mares from 1 farm in the ETA group had a history of application of pesticide to the pasture during the 4-week period prior to the index abortion.

Cherry trees inside or overhanging the pasture in which the mare resided during the 4-week period prior to the index abortion did not differ significantly between the ETA (107/121, 88%) and LOW-IMPACT (60/78, 77%) groups. Although cherry tree seedlings in the pasture was reported more frequently for the ETA group (23/106, 22%) than for the LOW-IMPACT group (8/78, 10%), the values did not differ significantly. Other fruit trees inside or overhanging the pasture in which the mare resided during the 4-week period prior to the index abortion did not differ significantly for the ETA (5/121, 4%) and LOW-IMPACT (8/83, 10%) groups.

Cherry trees outside the pasture in which the mare resided during the 4-week period prior to the index abortion did not differ significantly for the ETA (111/121, 92%) and LOW-IMPACT (70/83, 84%) groups. Other fruit trees outside the pasture in which the mare resided during the 4-week period prior to the index abortion was similar for the ETA (79/121, 65%) and LOW-IMPACT (52/83, 63%) groups. Although the proportion of mares in the ETA group that resided in pastures in which deciduous trees were stripped of their leaves during the spring of 2001 was greater than that of the LOW-IMPACT group (56/118 [47%] and 25/78 [32%], respectively), this finding did not differ significantly between groups. Although mares in the LOW-IMPACT group (15/83, 18%) were more likely to have trees in or around the pasture that were treated with pesticides during the 4-week period prior to the index abortion than were mares in the ETA group (9/121, 7%), the values did not differ significantly.

Vaccinations and anthelmintics

The ETA and LOW-IMPACT groups did not differ significantly in the frequency of administration of any vaccine or anthelmintic during the 4-week period prior to the index abortion.

Wildlife

Except for seeing deer or elk on the premises, none of the species of wildlife were associated with an increased risk of mares having ETA. During the preceding year, mares in the ETA group were significantly ($P = 0.047$) more likely to be from farms where deer or elk were seen frequently, compared with the observation of deer or elk at farms of mares in the LOW-IMPACT group (Table 1).

Eastern tent caterpillars

Several associations between exposure to eastern tent caterpillars and ETA were identified (Table 1). In general, caterpillar burdens were higher during 2001 than during 2000, although the proportion of mares in the ETA group for which it was reported that no caterpillars were observed during 2000 was smaller than the proportion of mares in the LOW-IMPACT group **(Table 2)**. However, these values did not differ significantly.

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The burden of caterpillars during 2001 was significantly ($P = 0.038$) more likely to be heavy at farms for mares of the ETA group than for mares of the LOW-IMPACT group. Because none of the mares in the ETA group were from farms described as having low numbers of caterpillars, it was necessary to combine the categories of low burden and moderate burden of caterpillars to compute an OR. Caterpillars were observed in trees in or around the pasture of all mares in the ETA group for which these data were known (107/107), compared with only 53 of 78 (68%) mares in the LOW-IMPACT group for which these data were known. Again, because of complete separation (ie, none of the mares in the ETA group were from farms that did not have caterpillars in trees), it was not possible to calculate an OR. Ignoring the correlation among observations, this observation was significant ($P < 0.001$), indicating this finding would likely have been significant if analysis had accounted for correlation among observations. Although the values did not differ significantly, more mares in the ETA group were from farms on which caterpillars were observed in pastures during 2001 (100/121, 83%), compared with mares in the LOW-IMPACT group (50/80, 62%). The percentage of pastures that had evidence of caterpillars during 2001 was significantly ($P = 0.012$) greater for the ETA group (median, 100% of pastures; range, 20 to 100% of pastures) than for the LOW-IMPACT group (median, 50% of pastures; range, 0 to 100% of pastures). When this factor was considered as a dichotomous categoric variable (caterpillars were seen in 100% of pastures; yes or no), the ETA group was significantly ($P = 0.012$) more likely than the LOW-IMPACT group to have had caterpillars seen in all pastures (Table 1).

Multivariate analysis

Those variables associated with a mare being in the ETA group with a value of $P < 0.10$ were included in multivariate modeling. Only 2 variables (feeding hay in pasture and amount of white clover in pasture that was larger than usual during the 4-week period prior to the index abortion) remained significantly associated with a mare being in the ETA group after analysis by use of multivariate methods. Two multivariate models, however, were considered (a model with just these 2 variables, and another model that included these 2 variables as well as the caterpillar-exposure variable that remained most strongly associated with a mare being in the ETA group; **Table 3**).

Table 2—Data regarding exposure to eastern tent caterpillars for mares in the ETA group and mares in the LOW-IMPACT group

Variable	ETA group	LOW-IMPACT group
Concentration of caterpillars observed during 2000		
Heavy	0 (0%)	5 (6%)
Moderate	43 (37%)	20 (24%)
Low	60 (52%)	33 (40%)
None	13 (11%)	25 (30%)
Concentration of caterpillars observed during 2001		
Heavy	106 (91%)	52 (63%)
Moderate	10 (9%)	21 (25%)
Low	0 (0%)	10 (12%)
None	0 (0%)	0 (0%)

Comparison of ETA group with FARM group—Data were compared between the ETA group and FARM group.

Farms and residency

The ETA group comprised 121 mares from 25 farms (median, 5 mares/farm; range, 3 to 6 mares/farm). The FARM group comprised 120 mares from these same 25 farms (median, 5 mares/farm; range, 3 to 6 mares/farm). The ETA and FARM groups did not differ significantly in the number of horses sharing the pasture or the amount of land in each pasture in which mares were maintained. Although seemingly similar for both groups, duration of residence in Kentucky was significantly ($P = 0.036$) shorter for mares in the ETA group than for mares in the FARM group (**Table 4**). However, duration of residence at the farm did not differ significantly between mares in the ETA group (median, 2 years; range, 0.08 to 13 years) and mares in the FARM group (median, 2 years; range, 0.2 to 25 years).

Age, parity, and breed

Although seemingly similar for both groups, the age of mares in the ETA group was significantly ($P = 0.032$) less than that of mares in the FARM group (Table 4). Although seemingly similar for both groups, the parity of mares was significantly ($P = 0.026$) lower for the ETA group than for the FARM group. All mares in both groups were Thoroughbreds.

Breeding history

The last breeding date for the ETA group ranged from February 10 to June 4, 2001 (median, February 28). The last breeding date for the FARM group ranged from February 10 to June 5, 2001 (median, March 14). Percentage of mares at the farm bred before April 1, 2001, for the ETA group (median, 39%; range, 7.5 to 77%) was identical to that of the FARM group (median, 39%; range, 7.5 to 70%). All of the mares in the FARM group and all but 1 of the mares in the ETA group were bred in Kentucky. Mares in the ETA group (26/116, 22%) were not significantly more likely than mares in the FARM group (19/116, 16%) to have a history of abortion during the preceding 5 years.

Feeding practices

The ETA and FARM groups did not differ signifi-

Table 3—Results of 2 multivariate models that compared data for 121 mares in the ETA group and 83 mares in the LOW-IMPACT group

Model	Variable	OR	95% CI	*P* value
1	Hay fed in pasture	27.8	2.8, 279.1	0.005
	Amount of white clover in pasture was larger than usual during the 4-week period prior to abortion	9.0	1.7, 47.8	0.010
2	Hay fed in pasture	34.1	3.0, 389.8	0.004
	Amount of white clover in pasture was larger than usual during the 4-week period prior to abortion	8.4	1.3, 52.1	0.023
	Caterpillars observed in all pastures	4.8	0.7. 33.6	0.116

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Table 4—Estimated OR obtained by use of conditional logistic regression for comparison of data for 121 mares in the ETA group and 120 mares from the same farms as the ETA mares but that did not abort and that were matched (± 14 days) on the basis of breeding date with mares in the ETA group (FARM group)

Variable	ETA group	FARM group	OR (95% CI)*	*P* value
Duration of mare's residence in Kentucky (years)				
Median	4	4	0.9 (< 0.9, < 1.0)	0.036
Range	0.1–13	0.2–25	1	NA
Age of mare (years)				
Median	8	9	0.9 (< 0.9, < 1.0)	0.032
Range	3–19	4– 25	1	NA
Parity of mare (No. of foals)				
Median	2	2	0.9 (0.8, < 1.0)	0.026
Range	0–11	0–18	1	NA

cantly for any covariates examined pertaining to feeding practices (eg, types or amounts of concentrate, hay, or supplements fed to mares; where hay was fed).

Watering practices

The ETA and FARM groups did not differ significantly with regard to source of water or types of water containers or vessels.

Stabling or housing practices

The ETA and FARM groups did not differ significantly for any covariates pertaining to stabling or housing practices (eg, number of hours in a stall daily during each of the 4 weeks prior to the index abortion, pasture intake).

Pasture characteristics

Mares in the ETA group did not differ from mares in the FARM group for any of the covariates pertaining to pasture characteristics (eg, type of grass in pasture, abnormal growth of white clover, detection of hemlock in pasture). The ETA and FARM groups did not differ significantly in the frequency of finding cherry trees inside or overhanging the pasture in which the mare resided during the 4-week period prior to the index abortion, frequency of cherry tree seedlings being in the pasture, frequency of finding other fruit trees inside or overhanging the pasture in which the mare resided during the 4-week period prior to the index abortion, frequency of finding cherry trees outside the pasture in which the mare resided during the 4-week period prior to the index abortion, frequency of finding other fruit trees outside the pasture in which the mare resided during the 4-week period prior to the index abortion, proportion of mares that resided in pastures in which deciduous trees had been stripped of their leaves during the spring of 2001, or frequency of having trees in or around the pasture that were treated with pesticides during the 4-week period prior to the index abortion.

Vaccinations and anthelmintics

The ETA and FARM groups did not differ significantly in frequency of administration of any vaccine or anthelmintic during the 4-week period prior to the index abortion.

Wildlife

As expected on the basis of the design of our study, the ETA and FARM groups did not differ significantly in the frequency of observation of any species of wildlife at the premises during the 12-month period prior to data collection.

Eastern tent caterpillars

Caterpillars were ob-served in trees in or around the pasture of all mares in the ETA (n = 107) and FARM (106) groups for which these data were known. Mares in the ETA group were equally likely to have been in pastures in which caterpillars were observed during 2001 (100/121, 83%) as mares in the FARM group (99/120, 83%). Other variables associated with caterpillar exposure were not examined, because they were farm-level variables, and mares in the ETA and FARM groups were matched on the basis of farm.

Multivariate analysis

Age, parity, and duration of residence in Kentucky were examined by use of multivariate models. Models that included the terms age and duration of residence in Kentucky, age and parity, and parity and duration of residence were examined. None of the variables remained significantly associated with a mare being in the ETA group. Put another way, after accounting for effects of age, neither parity nor duration of residence was significantly associated with a mare being in the ETA group. Exploratory data analysis indicated that age, parity, and duration of residence in Kentucky were each coassociated.

Discussion

A number of variables were significantly associated with development of ETAs attributed to MRLS when mares with ETAs at farms considered to be severely impacted by MRLS (ETA group) were compared with mares that did not abort from farms that were considered to have been less impacted (LOW-IMPACT group). Of these, the most important factors appeared to be hay fed in pasture, an amount of clover in pasture that was larger than usual, and finding eastern tent caterpillars in pastures.

The association of fetal loss with feeding hay in pasture (relative to feeding hay exclusively in a stall) likely indicated an association of fetal loss with exposure to some toxic agent or agents in pastures. That is, feeding hay in pasture was an indicator of exposure to pasture. It is possible that horses fed hay in pasture (presumably on the ground) had greater contact with the causal agent or agents found in pastures. Alternatively, it is possible that mares at farms that were fed hay in pasture were at greater risk because of some unmeasured management practices or environmental conditions at the farm.

Fetal loss was associated with an amount of white clover that was considered to be larger than usual in the pasture during the 4-week period prior to the estimated time of abortion. Although it is possible that there was a toxic principle in the white clover, it is also possible that the rapid growth of white clover was merely a marker for the toxic agent or agents or environmental conditions that favored elaboration of the toxic agent or agents to which affected mares were exposed. Although the association of rapid growth of white clover with fetal loss was strong, it was not absolute; almost a fourth of the affected mares were not exposed, and approximately a third of mares from low-impact farms

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were exposed. This finding may be important, however, because rapid growth of white clover with unusual climactic conditions similar to those that occurred during 2001 could presage another outbreak and indicate the need to implement measures to prevent MRLS.

The association between fetal loss and caterpillars was included in 1 of the final multivariate models, despite the fact it did not remain significant, because a number of factors pertaining to caterpillars (heavy burden of caterpillars during 2001, evidence of caterpillars in trees around the pasture) were associated with being in the ETA group, and because investigators observed an association between caterpillar burden and fetal losses in a farm-based epidemiologic study.[1]

The causal role of caterpillars (or any other exposure) could not be determined from the study reported here. It is possible that the caterpillars contained a toxic agent that contaminated pastures via their excretions or secretions, or exposure may have resulted from consumption of caterpillars by horses in pastures. If true, this would indicate that application of pesticides to reduce or eliminate caterpillars from the environment of horses could prevent cases of MRLS. Alternatively, caterpillars may have acted as fomites and mechanically transmitted a toxic agent that contaminated pastures, or excretions by caterpillars may have acted synergistically with some agent in pastures such that the caterpillars amplified the effects of exposure to that agent. Finding caterpillars in pastures may have been a marker for some other farm-level factor such as management practices, farm conditions (farms with a large number of trees), or environmental conditions that predisposed to MRLS. This contention was supported by the finding that, after adjusting for the 2 covariates most strongly associated with MRLS (ie, hay fed in pasture and an amount of white clover that was larger than usual in pastures during the 4-week period prior to abortion), the association with caterpillars was no longer significant. However, statistical power to detect this difference may have been limited by the sample size. Furthermore, because exposure to caterpillars occurred primarily in pastures, the association between fetal losses and caterpillars may have been diluted by simultaneous consideration of other indicators of pasture exposure such as feeding hay in pasture. Finally, the association with caterpillars may have reflected a form of recall or information bias. Given that the study reported here was conducted following reports linking caterpillars and cherry trees to MRLS, representatives of farms that had many fetal losses may have been more inclined to recall or report heavier exposure to caterpillars than were recalled by representatives of farms that had fewer fetal losses. The likelihood of this bias is diminished somewhat by the finding that an association between fetal losses and exposure to cherry trees was lacking.

Although caterpillars were associated with ETAs, they were not associated with late-term abortions in another study[2] conducted by our group. The reason for this apparent discrepancy is unclear, but there are explanations. First, it is possible that the conditions that predisposed to ETAs resulted in the production of multiple causal agents that caused differing clinical syndromes (ETAs, late-term abortions, pericarditis, and uveitis). Thus, caterpillars may have played an important role for some of the clinical conditions but not the others. Alternatively, caterpillars might have modified or disseminated an agent that was in or around the pastures of affected mares. Finally, both studies on ETAs and late-term abortions pointed to various indicators of exposure to pastures as factors that increased the risk of fetal loss. Exposure to caterpillars likely occurred in pastures, such that the caterpillars may have been another indicator of pasture exposure that was stronger in the study reported here than in the study on late-term abortions. Because the study designs and populations differed for this study and the study of late-term abortions, discrepant results would not be unexpected. For example, stabling practices for mares in early gestation may differ from those for mares in late gestation such that an association of stabling practices with abortion as a result of exposure to an agent in pasture might be less apparent in a study of mares in early gestation, compared with mares in late gestation.

Several factors that were significantly associated with fetal loss were not included in the final model, but they were significant in bivariate analysis. Mares in the ETA group were almost 3 times more likely to have had an abortion during the previous 5 years than were mares in the LOW-IMPACT group. Although the importance of this observation is unknown, it may indicate a predisposition to abortion or fetal loss among affected mares. This finding also may indicate closer monitoring or greater reporting of adverse reproductive outcomes for affected farms than for unaffected farms. Alternatively, the observation may have occurred by chance alone.

Pericarditis associated with MRLS was significantly more likely to have been reported at the farms of mares in the ETA group, compared with farms of mares in the LOW-IMPACT group. This corroborates other observations by our group that the epidemic of fibrinous pericarditis was epidemiologically and temporally linked to fetal losses associated with MRLS.

Rapid growth of some form of plant life that was in an amount larger than usual during the 4-week period prior to the index case of abortion was significantly more likely for the ETA group than for the LOW-IMPACT group. The most commonly reported plant was white clover. Rapid growth of plants may have been an indicator of other environmental conditions that predisposed mares to MRLS, a contributing factor for MRLS (ie, provided conditions favorable for elaboration, persistence, or dissemination of some toxic agent or agents), or a causal factor of MRLS (eg, elaboration of phytoestrogens or cyanogens by plant life). The finding that an excess amount of white clover in pasture during April and May 2001 relative to the same months for 2000 was associated with fetal loss is likely to have the same explanations as those proposed for a rapid growth of white clover in the pasture during the 4-week period prior to the index abortion (ie, the white clover may be a causal factor, a cofactor, a confounding factor, or a coincidental factor).

The finding that sighting of deer or elk during the year prior to the time of data collection was significantly more common for the ETA group than the LOW-IMPACT group was unexpected. It is possible that environmental conditions that favored deer or elk (eg, farms with more trees or wooded areas at or adjacent to the farm) were related to factors that increased risk of fetal

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losses. Alternatively, farms where deer and elk were observed may have been spatially located in areas of higher risk. Finally, the observation may have been spurious (confounded) or occurred by chance alone. To the authors' knowledge, elk are not found in Kentucky. Elk were included in the question to be consistent with a question posed by other epidemiologic investigators.

Use of mares from the same farm as affected mares to serve as control animals revealed that none of the factors other than those related directly or indirectly to age of mare were significantly associated with fetal loss. The clinical importance of the difference in age for mares that had fetal loss relative to control mares from the same farm is questionable, because median and ranges of ages were quite similar, and the resultant OR was relatively small. One possible explanation of these associations was that maiden mares (ie, mares that were never bred) were over-represented in the ETA group, assuming that maiden mares were younger than mares that had been bred. Recently, maiden mares and barren mares (mares that had been bred the preceding year but that did not become pregnant to that breeding) at 4 farms in central Kentucky were reported to be at increased risk of MRLS-associated early fetal loss.[5] Mares were not specifically categorized as maiden or barren in the study reported here. Although the proportion of mares with parity of 0 was greater in the ETA group than the FARM group, the values did not differ significantly.

Although the factors that differed significantly between the ETA and FARM groups may not have been clinically meaningful (unless maiden mares were implicated), the lack of significant associations had important implications. First, failure to identify factors differentiating affected from unaffected mares indicated that exposures within a farm were similar for affected and unaffected mares and that farm-level epidemiologic studies and studies comparing mares from affected and less affected farms are necessary and appropriate for investigation of MRLS. Second, it indicated that a temporal study of ETAs was indicated, because mares in both control populations were closely matched with affected mares on the basis of breeding date. Although this prevented breeding date (ie, time of year) from confounding comparisons between mares from high-impact farms with those from farms that were least impacted, it precluded our ability to examine whether breeding date and gestational age were risk factors for the condition at high-impact farms. Conceivably, the most important factors at farms that were severely affected was pasture exposure among mares at a particular time of the year and at a particular stage of gestation.

Median age of the fetus when it was last documented as viable was 60 days. Only 3 fetuses had the last documentation of viability at < 30 days of age (16, 21, and 29 days, respectively). Only 3 fetuses had the last documentation of viability at > 90 days of age (120, 202, and 205 days, respectively). Although these 3 fetuses did not meet the criterion specified for ETA in this study (ie, fetal loss at ≤ 90 days of gestation), exclusion of data for the mares of these 3 fetuses did not alter results of the study.

Data in this study provided information for mares bred between February 10 and June 6, 2001. As mentioned, a temporal study is needed to define the period of greatest risk of MRLS-associated fetal loss. Assuming that exposure to the cause of MRLS was greatest during a specific period, it is possible that mares were included in this study that were not at risk of fetal loss because they were not bred during the period of risk for MRLS or the fetus was not at a susceptible gestational age during the period of risk. The net effect of this misclassification would have been to bias our results away from identifying significant associations (ie, biasing OR toward 1).

An assumption of our study was that farms with the highest rates of pregnancy losses or largest changes in pregnancy losses in 2001 relative to 2000 were affected by MRLS and that farms with lower rates were less impacted by MRLS. The validity of this assumption cannot be assessed, because a definitive diagnosis for fetal losses attributable to MRLS is lacking. Thus, it is possible some farms classified as least impacted by MRLS were, indeed, affected more heavily by MRLS (ie, ETAs or other conditions associated with MRLS). The net effect of this misclassification would have been to bias our results away from finding significant differences between mares affected by MRLS and mares not affected by MRLS.

Because breeding dates were similar for mares in the ETA group and mares in the other 2 groups, it is improbable that substantially fewer mares from the less impacted farms were susceptible during a critical time of risk for MRLS. Mares in the LOW-IMPACT group, however, were bred, on average, 2 weeks after mares in the ETA group, and it is possible that there were fewer susceptible pregnant mares at the low-impact farms. It should be mentioned that categorization of farms was based on the distribution of rates of pregnancy losses and was made prior to analysis of any data regarding predictors of outcome.

Our study did not identify a cause of ETAs caused by MRLS. Characteristics of pastures and exposure to pastures were important risk factors for MRLS. It is likely that exposure to pastures was a risk within the constraints of a particular time period during which there were peculiar climactic and environmental conditions in the spring of 2001. Specific recommendations for prevention of MRLS cannot be made on the basis of this study alone; however, reducing exposure to pastures (particularly those with white clover) and caterpillars during similar environmental conditions as those that occurred during the spring of 2001 could decrease the risk of MRLS.

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[a]Hagyard-Davidson-McGee Associates, Lexington, Ky.
[b]Rood & Riddle Equine Hospital, Lexington, Ky.
[c]A copy of the questionnaire is available on request.
[d]S-PLUS 6.0, Insightful Inc, Seattle, Wash.

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