Session 6

Experimental Replication of Mare Reproductive Loss Syndrome

Chairperson: Dr. Nancy Cox, Associate Dean for Research, College of Agriculture, University of Kentucky

Induction of Mare Reproductive Loss Syndrome by Directed Exposure of Susceptible Mares to Eastern Tent Caterpillar Larvae and Frass

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RESEARCH INTO THE CAUSE OF MARE REPRODUCTIVE LOSS Syndrome (MRLS) has been ongoing since the disease was recognized in the spring of 2001. We report here results of a collaborative project involving the Department of Entomology, the Maxwell H. Gluck Equine Research Center, and the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC). An intensive survey of horse farms in Central Kentucky in 2001 under the leadership of Dr. Roberta Dwyer found a strong correlation between the presence of eastern tent caterpillars (ETC) on farms and the incidence of MRLS. This project was designed to determine if the association was simply a *correlation* or if tent caterpillars were *causally* associated with MRLS. Therefore, these studies were also designed to mimic on-farm conditions by exposing pregnant mares to ETC in the fields on which the mares grazed.

Methods

Two experiments were performed. In Experiment 1, pregnant mares (between approximately 40 days and 7 months gestation) were exposed to increasingly high levels of ETC and their frass (n = 10 mares) or increasing levels of frass (n = 9 mares). In the control group (n = 10 mares), mares were handled identically to the experimental treatments, but attempts were made to minimize exposure to ETC larvae and frass. Treatments were administered in a randomized split plot design with animals subjected to experimental conditions in 16 x 16 ft pens in 20 x 200 ft treatment plots for 6 hours/day over two 10-day periods of exposure. The experiment was designed such that plot size was reduced with consequent increase of exposure over the course of the treatment. When not in pens, mares were pastured communally in adjacent areas. Mares were examined by manual palpation and ultrasonography once a week.

In Experiment 2, pregnant mares (between approximately 40 days and 6 months gestation) were divided into three treatment groups of eight mares each. Treatments were increasing levels of starved ETC, increasing levels of ETC frass, or control (no treatment added to the pasture plots). The ETC were collected two to three weeks prior to the experiment and had not been fed; thus, they would deliver little or no frass to the treatment plots. Additionally, ETC used in this study included mature, wandering-stage caterpillars. The ETC frass was collected two to four weeks prior to the experiment and was stored frozen. The design was to separate potential effects of the caterpillars themselves from those of the caterpillar frass. For 6 hours each day over a 10-day period, mares were placed individually in 16 x 16 ft pens, on plots as described for Experiment 1. Pasture plots for Experiment 2 did not overlap areas of the pasture previously used for Experiment 1. Turn-out fields for the mares when they were not in the experimental pens was the same for Experiments 1 and 2. All mares were examined daily, beginning prior to the onset of treatment and continuing for 10 days after the last treatment. Mares were then examined weekly for an additional four weeks.

Results and Discussion

In Experiment 1, pregnancy losses were 7 of 10 mares deliberately exposed to ETC and frass, 7 of 9 mares exposed to frass only, and 3 of 10 in the control group. Diagnostic evaluations of the mares and recovered fetuses were consistent with signs observed for fetal losses suffered in MRLS in 2001. In Experiment 2, pregnancy losses were 3 of 8 in the starved ETC group, 0 of 8 in the frass group, and 1 of 8 in the control group. Ultrasound image echotexture of the fetal fluids, as well as pathologic and bacteriologic findings, were consistent with MRLS-type abortions for all losses that occurred in both experiments.

These studies provided the first experimental evidence that ETC induce pregnancy loss in horses and were the first to reproduce the syndrome under experimental conditions. Furthermore, they showed that exposure to ETC and frass in Experiment 1 induced losses under conditions that mimicked field exposure. Experiment 1 was formulated with the objective of reproducing the syndrome in advance of its appearance in the field and thereby to provide timely information to farm managers and veterinarians in the region. Losses in this experiment were advanced relative to the occurrence of MRLS elsewhere in Central Kentucky in 2002, and preliminary results of the study were released in late April and reinforced recommendations to minimize exposure of susceptible mares to ETC. The four mares in the control groups that lost their pregnancies (three in Experiment 1 and the single loss in Experiment 2) were all in close proximity to one plot that

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received ETC as its experimental treatment. Monitoring of all pens for ETC indicated that the ETC containment system failed in some plots and resulted in elevated ETC levels in adjacent control plots. Mares in the control plots that suffered losses were all adjacent to ETC treatments and adjacent to the plot in which partial failure of the containment system was indicated. Therefore, it is highly likely that mares in these control treatments were exposed to ETC larvae in Experiment 1 with this exposure causing the observed losses. Based on these studies, we conclude that ETC larvae are *causally* associated with MRLS, but involvement of ETC frass remains unclear.

Gastric Administration of Eastern Tent Caterpillars Causes Early Fetal Loss in Pregnant Mares

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GASTRIC ADMINISTRATION OF STARVED EASTERN TENT CATerpillars (ETC) resulted in early pregnancy loss in the mare. The study involved three groups of mares. One group received starved caterpillars, one group received caterpillar frass, and one group served as control. Four of five mares administered caterpillars lost pregnancies within 8 to 13 days subsequent to the first dose of ETC. No control mares or mares that received frass aborted.

Materials and Methods

The study design consisted of three groups of five mares each. Mares were administered their respective treatment by nasogastric tube for 10 days. Group 1 mares served as controls and received 50 ml of water, Group 2 mares received 2.5 g of stored frass diluted in 50 ml water, and Group 3 mares received 50 g of crushed fresh ETC mixed in 50 ml of water. Mares were housed in stalls with no exposure to grass beginning 12 days before they were given any treatment and remained in stalls for the entire experimental period. Mares were walked twice daily to provide exercise. Mares were between 38 and 88 days of gestation on day one of treatment.

Results

Early fetal losses (EFL) were observed in 4 of 5 Group 2 mares. No control mares or mares receiving frass aborted. The mares that aborted were 49, 64, 70, and 96 days of gestation. *Alpha streptococcus* was cultured from one aborted fetus, while *Serratia* sp. was cultured from the remaining three fetuses. Neither pericarditis or ophthalmologic disease was observed. There were no significant changes in serum chemistries or leukograms. One of the aborting mares exhibited signs of abdominal pain two days prior to abortion.

Discussion

In the spring of 2001, a severe illness affecting pregnant mares occurred in Central Kentucky. The condition occurred across breeds and under a variety of management conditions. A variety of theories regarding the causative factor or factors has been proposed, one of which is the caterpillar or its frass. An epidemiologic survey identified a relationship between high numbers of ETC and EFL (1). A field study (B. Webb et al., this proceedings) suggested that ETC and/or frass were causally related to MRLS. This study confirms that ETC can cause EFL. The toxic agent (biological or chemical) is yet to be elucidated.

Conclusion

The investigators concluded from this study that ETC can cause EFL in the pregnant mares and that "stored" frass does not cause EFL. The study did not define the toxic component of the caterpillar responsible for the EFL.

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Experimentally Induced Mare Reproductive Loss Syndrome Late Fetal Losses with Eastern Tent Caterpillars

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DURING 2001, CENTRAL KENTUCKY HAD AN EPIDEMIC OF early and late fetal losses (EFL/LFL), which was together called Mare Reproductive Loss Syndrome (MRLS). The LFL began in the last week of April, peaked on May 5, and declined rapidly. EFL was identified on April 26 and had a similar course and ultimately totaled about 1,500 cases. A total of 450 LFL cases were submitted to the University of Kentucky Livestock Disease Diagnostic Center (UKLDDC) for detailed pathological examination during a period of two months (1). Concurrent with the epidemic was a local population explosion of eastern tent caterpillar (ETC), *Malacosoma americanum*.

Experiments conducted by Webb et al. (2) and Bernard et al. (3) have established a causal role for ETC in EFL. The necropsy examination of LFL demonstrated lesions not observed in EFL and bacteriological findings that can be compared to EFL. If reproduced experimentally, these observations will help in defining the specific pathogenesis, etiological agent, and clinical picture. Hence, a study was undertaken to evaluate the ability of ETC to reproduce abortion in late and midterm pregnancies.

Materials and Methods

Eleven pregnant mares obtained from a commercial nurse mare operation in late stage of pregnancy (9 to 11 months of pregnancy) were selected for the experiment. Mares were divided into two groups, six in the treatment group and five in the control group. Mares in the treatment group were administered 50 g of ETC collected from the Upper Peninsula of Michigan fed on wild cherry tree leaves mixed in 70 ml of normal saline for 9 days. The control group mares received 120 ml of normal saline for 9 days. All of the mares were confined to the stall during the entire period of experiment. They were fed hay and had constant access to clean drinking water. Mares were walked once daily to provide exercise. Blood was collected from the jugular vein 5 days prior to experiment and all 9 days of the experiment and every other day after the experiment for 10 days. Complete blood count and biochemical assays were performed within 2 to 3 hours of collection with an auto analyzer. Blood was collected aseptically from all the mares prior to experiment and also during the entire period of 9 days of experiment for bacterial culture. The serum concentration of progesterone and estrogen were estimated on the day before the experiment and on the day of abortion.

All the mares were monitored prior to the experiment by rectal ultrasonography and on days 3, 5, 7, and 9 by transabdominal ultrasonography for fetal heart rate, placental thickness, and appearance of fetal fluid.

Results

Five of the treatment mares aborted during the experiment, and the sixth mare aborted on day 15 from the first day of exposure. A complete necropsy was done on all aborted fetuses and placentas. A uterine biopsy was taken from all the aborted mares on the day of abortion. FATs were done on pooled tissue samples of all fetuses for *Leptospira* species and EHV. The first abortion occurred between 56 to 69 hours, and the last abortion at 357 hours on the fifteenth day. All the placentas had intact cervical stars, and two had tears of the allantochorion. The weight of the placentas ranged from 5 to 10 pounds. The length of the umbilical cords ranged from 55 to 82 cm. All the placentas' chorionic surface had a pale brown color compared to the dark red color seen on the chorionic surface of fresh placentas. The weight of the fetus ranged from 35 pounds to 82 pounds.

Enterobacter sakazakii (two fetuses), *Serratia marcescens*, and *Enterococcus* species (two fetuses) and *Enterobacter cloacae* (two fetuses) were isolated from multiple organs of the aborted fetuses. There was no significant difference in the complete blood count, serum biochemistry, coagulation factors, and ammonia levels between the treatment group and the control group. Detailed histopathological examination of the fetus and placenta showed hemorrhage and congestion of adrenals (6 of 6 fetuses), congestion of liver (5 of 6 fetuses), congestion of kidney (5 of 6 fetuses), hemorrhage in epicardium (3 of 6 fetuses), congestion of thymus (6 of 6 fetuses), congestion of spleen (5 of 6 fetuses), endometritis (6 of 6 mares), bronchopneumonia (1 of 6 fetuses), hepatic necrosis (1 of 6 fetuses), placentitis (1 of 6 fetuses), amnionitis (1 of 6 fetuses), and

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funisitis (1 of 6 fetuses). The fetal heart rate was increased in all fetuses on days prior to the day of abortion. FATs done on the pooled tissue samples of all aborted fetuses were negative for EHV and *Leptospira* species. Blood culture for bacteria yielded no bacteria during the entire period of experiment. There were no significant observations in the fetal fluid or placental thickness. All the fetuses showed significant increase in fetal heart rate before they aborted or were diagnosed dead *in utero*. The serum concentration of progesterone from the day before exposure of caterpillars, when compared to the day of abortion, had a drastic drop in concentration. The serum concentration of estrogen on the day of abortion was significantly lower than the day before the exposure of caterpillars.

Discussion

One consistent finding for all abortions was an intact cervical star similar to the gross finding in naturally occurring cases of LFL. Also, the chorionic surface of 4 of 6 allantochorions had a light brown yellow appearance, indicating placental detachment that remained *in utero* for sufficient time to cause fetal death. The ultrasound examination of the fetal heart rates showed an increase in heart rate indicating the fetuses may have been under stress due to hypoxia. Placenta detachment may have occurred leading to physiological effects consistent with fetal hypoxia. The fetus of the mare that aborted on day 15 had fetal diarrhea, which likewise underscores a possible connection to fetal hypoxia. Congestion and hemorrhage noted in fetal tissues may also be seen as indication that fetal hypoxia is a factor in the pathogenesis of MRLS. The fetus of the mare aborted on day 15 had fetal diarrhea and funisitis similar to the fetuses in naturally occurring cases of LFL (4). Enterobacter species, *Serratia marcencens*, and. Enterococcus species bacteria isolated in these cases were also isolated in naturally occurring cases of LFL (5).

The dose of ETC used for this experiment was based on the dose used in the EFL experiments (2,3). Abortion occurred earlier in this experiment as compared to the EFL experiment by Bernard et al. (3) in which the first abortion occurred on day 8. The first abortion in this study was observed at 69 hours post administration of ETC. The apparent difference in the early onset of abortion may be due to ETC in this experiment being fed fresh cherry tree leaves until the day of preparation, while in the EFL study by Bernard et al. (3), the ETC had been kept away from cherry tree leaves several days prior to preparation and administration. This difference in the onset of abortion/fetal death suggests that the caterpillars fed cherry tree leaves have more abortifacient potential when compared to starved caterpillars. Another fact to consider is that metabolically active ETC may be producing an abortifacient agent in much higher quantities compared to starved ETC.

Conclusion

This experiment indicates that dosing late-term pregnant mares with a preparation of ETC that had consumed wild cherry trees leaves causes abortion consistent with MRLS. Caterpillars were collected from the Upper Peninsula of Michigan and were shipped to Lexington for the experiment, indicating that ETC from other states have the potential to induce LFL in horses.

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