

AN UPDATE ON MARE REPRODUCTIVE LOSS SYNDROME (MRLS) IN KENTUCKY

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During April and May of 2001 the pregnant mare population of central Kentucky, comprising multiple breeds of horses, experienced a high level of fetal loss. They included early (40-100 day) fetal losses (EFL) plus late term abortions and the birth of weak foals, referred to as late fetal losses (LFL). With no immediate explanation for the cause it was identified as Mare Reproductive Loss Syndrome (MRLS). Also reported during the month of May were an unusually high number of cases of unilateral uveitis and pericarditis among horses of all breeds, ages and sexes.

Despite intensive investigation the cause of MRLS was not identified during 2001 although epidemiological studies undertaken during the summer identified high and low risk factors associated with MRLS. High risk factors included the presence of the wild black cherry tree and high to medium populations of the eastern tent caterpillar (*Malacosoma americanum*).

Prior to the start of the 2002-breeding season a series of recommendations to control MRLS including a monitoring program on selected farms was published by the University of Kentucky's College of Agriculture. Several research projects on MRLS were also approved, to be undertaken by scientists within the College in 2002.

Commencing the last week of April and early May 2002 cases of MRLS were reported on a number of farms in central Kentucky albeit at a reduced level compared to 2001.

Clinical and Pathological Description of MRLS

During the last week of April 2001 veterinarians specializing in equine reproductive practice began examining pregnant mares over 60 days of gestation utilizing ultrasonography to determine the sex of the fetus. They reported an unusually high number of fetuses were not viable, lacking a heart beat, plus the presence of cloudy, flocculent material in the allantoic and amniotic fluids. Subsequent examination of these cases confirmed the fetus was no longer present. At the same time the Livestock Disease Diagnostic Center (LDDC) in Lexington reported a significant increase in the number of late term abortions submitted for post mortem examination. The mare history indicated no premonitory signs. In many cases delivery was characterised as 'red bag' indicating the allantochorion was present and passed with the fetus.

Approximately 550 LFL were submitted from 17 breeds of horses distributed over 32 counties in the state of Kentucky. Pathological lesions associated with these cases have been described by Williams (2002, Mare Reproductive Loss Syndrome: Pathologic Findings. Equine Disease Quarterly, Volume 10, Number 4). They include inflammation of the umbilical cord (funitis) and amnion (amnionitis), pneumonia, fetal bacteremia and sometimes placentitis. The findings are suggestive of *in utero* fetal illness and distress but lack specificity to permit diagnosis of the inciting cause. Bacterial cultures of the fetus and placenta yielded beta hemolytic *Streptococci* spp. and/or *Actinobacillus* spp. in over 50% and approximately 20% of the cases respectively. Figures on the number of EFL are less precise with estimates ranging from 20-30% of the pregnant mare population of Kentucky. Registrations to the Jockey Club of foals born in 2002

will provide an accurate figure for the Thoroughbred population in the state. As of May 6, 2002 the Jockey Club reported 5088 foal registrations, a reduction of 23.4% compared to the same period in 2001. Kentucky has an annual foal crop of approximately 10,000 that represents 30% of North America's yearly Thoroughbred foal population.

In addition to the reproductive losses an increased number of newborn foals suffering acute respiratory distress were admitted to the local equine veterinary hospitals. These cases required intensive veterinary and nursing care and a number did not survive. Approximately 30 cases of unilateral uveitis or endophthalmitis involving hemorrhage and fibrin deposition were reported during May among horse of all breeds, ages and sexes. These cases did not respond to treatment leading to loss of sight in the affected eye. During the same time frame approximately 60 cases of pericarditis were reported again among horses of all breeds, ages and sexes. Dr Nathan Slovis of Hagyard-Davidson-McGee Associates reported that clinical signs included fever, lethargy, anorexia and muffled heart sounds. Ultrasound examination of the heart revealed a fibrin layer on the epicardium and an abundant amount of fluid in the pericardium. Approximately half of the cases were submitted for post mortem examination. Dr. David Bolin, pathologist at the LDDC confirmed that the majority of horses with pericarditis had some degree of peritoneal, pleural and pericardial effusion. The pericardial sac was markedly thickened. A thick shaggy layer of fibrin 0.5 to 1.5 cm thick covered the visceral surface of the pericardium and epicardium. Subsequently cases of both eye and heart conditions were included within the 'Case Definition' of MRLS.

During April and May 2002 an identical clinical and pathological picture re-emerged with cases of early and late fetal losses as well as cases of pericarditis and uveitis consistent with what was associated with MRLS in 2001. Numbers were significantly reduced as compared to 2001. A report on 3,089 mares examined by veterinarians in two large equine practices in Lexington over 42 days of gestation between April 30 and May 17 confirmed a pregnancy rate of 90% with fetal losses attributed to MRLS in the region of 8%. Normal losses during this period would range from 1-2%. LFL losses submitted to the LDDC attributed on examination to MRLS numbered 160 from April 28 to May 25.

Investigations into the Cause of MRLS in 2001

Preliminary observations indicated that reproductive losses occurred simultaneously over a wide area of central Kentucky on many different farms commencing during the latter part of April. This observation was more indicative of an environmental toxin as distinct from an infectious agent. Investigations by several laboratories have so far failed to implicate a primary disease pathogen. The isolation of opportunistic bacteria is considered to be a secondary invader. Initial investigations concentrated on the role of environmental toxins including mycotoxins, fungal endophytes, phytoestrogens and chemical compounds including cyanide. Analysis of fetal heart tissues submitted May 17, 2001 to the Toxicology Section, Laboratories of Veterinary Diagnostic Medicine, College of Veterinary Medicine, University of Illinois indicated low levels of cyanide or cyanide like compounds by the distillation-ion specific electrode method. This observation gave rise to the 'working hypothesis' that cyanide or cyanogenic compounds derived from leaves of the wild black cherry were delivered to mares by the eastern tent caterpillar (Harrison, 2001, Kentucky equine abortion storm and related conditions. Proceedings of the 105 Annual Meeting of the United States Animal Health Association, pp. 227-229). During late April and early May there was an unusually high emergence of the eastern tent caterpillar in central

Kentucky crawling on fence posts, pastures and buildings and filling water troughs and buckets. Field visits had identified the presence of cherry trees in or around pastures containing mares that had experienced MRLS. The majority of these trees containing 'caterpillar tents' had been denuded of leaves eaten by the caterpillars. The caterpillars had then left the trees crawling through pasture in search of a source of nutrition. Subsequent studies undertaken in the College of Agriculture's Departments of Entomology and Veterinary Science by Drs. Webb, Tobin and Harrison have to date failed to confirm cyanide as the causative agent of MRLS.

Numerous analyses of pasture and equine samples also failed to implicate the role of known mycotoxins, fungal endophytes, phytoestrogens and other chemical compounds.

The abnormal weather pattern through April 2001 in central Kentucky comprising low rainfall, high temperatures, punctuated by severe frosts in mid April stimulated an investigation as to the possible involvement of weather as a trigger factor for MRLS. Examination of weather patterns archived by the College of Agriculture's meteorologist Tom Priddy identified a similar pattern during April 1981. During 1980 and 1981 early fetal losses were reported on a number of farms in central Kentucky among mares bred in February and March of those years (report compiled by Dr. J.T. Bryans, Chairman of the Department of Veterinary Science, 1981). This finding provided strong circumstantial evidence that MRLS had occurred in central Kentucky prior to 2001.

Epidemiological Studies Undertaken to Investigate MRLS

On May 7, 2001 a questionnaire was faxed to 280 members of the Kentucky Thoroughbred Farm Managers Club to determine the extent and impact of MRLS, particularly EFL on farms in five counties around Lexington. From 158 responses received by May 9 it was estimated that 678 (21%) of 3294 mares had experienced EFL between 42 and approximately 80 days gestation. (Dwyer et. al, 2002, An epidemiological investigation of Mare Reproductive Loss Syndrome: Breaking ground on a new disease. Twentieth Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, pp. 44-47).

Following this preliminary survey an in depth field epidemiological study was organized based on a three tiered approach involving 133 farms, pastures on those farms and selected mare populations. (Dwyer et. al, 2002, An epidemiological field study of factors associated with a new disease: Mare Reproductive Loss Syndrome (MRLS); submitted for publication). Twenty-three volunteers conducted personal interviews of farm owners or managers during June 2001 using a prepared questionnaire previously forwarded to the farm. Data entry and analyses of this extremely comprehensive survey was performed by USDA APHIS personnel in Frankfort, Kentucky and the Centers for Epidemiology and Animal Health, Fort Collins, Colorado. Results indicated that a high incidence of EFL was associated with pregnant mares exposed to high or medium levels of eastern tent caterpillars and cherry trees, farms with a population of more than 50 mares, mares bred in February 2001 and exposure to water fowl.

Later in the year a number of case control studies were undertaken by Dr Noah Cohen of Texas A and M University and colleagues to identify risk factors associated with cases of pericarditis, EFL and LFL (Cohen et. al, 2002, Report to the Governor's MRLS Task Force). Their findings

indicated that exposure of mares to pasture predisposed to MRLS and also implicated the role of the eastern tent caterpillar.

A field study of pregnant mares exposed and not exposed to MRLS in 2001 were monitored through pregnancy to birth of the foal in 2002 by regular ultrasound examination of the fetus. The findings provided no evidence the syndrome exerted an abnormal effect on the fetus and on placental and foal weight at birth. (Pantaleon et. al, 2002; submitted for publication).

In June of this year a further questionnaire is to be sent to members of the Farm Managers Club seeking to identify farms which did and did not experience EFL in 2002 and the respective preventive measures that each farm had taken.

Farm Monitoring and Recommendations for the Prevention of MRLS

The monitoring program published by the University of Kentucky's College of Agriculture for managing the risk of MRLS in 2002 includes 13 farms selected as 'sentinels' on which pasture and mare (blood and urine) samples were obtained at intervals of two weeks from March 1 through June 30. Detailed records of animal and pasture histories were maintained and samples examined for a variety of environmental toxins. Where EFL or LFL cases occurred then additional samples from the mare were obtained and the fetus if found submitted to the LDDC for examination. The contingency measures to reduce the risk of MRLS included minimizing or eliminating the exposure of pregnant mares to the eastern tent caterpillar, keeping pregnant mares away from cherry trees, frequent clipping of pastures on which pregnant mares grazed and offering hay to horses at pasture.

Ongoing Research Projects

In the fall of 2001 the College of Agriculture established an in-house Research Program to identify the causes and means of prevention of MRLS. A list of these projects is provided in Table 1.

Table 1. MRLS Related Research Projects at the University of Kentucky.

Title	Principal Investigator
Preliminary Observations on Insecticide Safety with Broodmares	Dr. K. McDowell
MRLS: Are Estrogens or Antiestrogens Involved?	Dr. K. McDowell
Evaluating Novel, Reduce-risk Insecticides for Managing Eastern Tent Caterpillar Outbreaks on Horse Farms	Dr. D. Potter Dr. L. Townsend
Eastern Tent Caterpillar Feeding in Horse Pastures: Behavioral and Ecological Aspects	Dr. L. Rieske-Kinney
Assessment of Direct and Indirect Interactions between Eastern Tent Caterpillars and Mares as Contributing Factors to Mare	Dr. B. Webb

Reproductive Loss Syndrome

Investigations of Mycotoxins as a Possible Cause of Mare Reproductive Loss Syndrome	Dr. K. Newman
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Evaluation of the Role of Cyanide and its Metabolites in Mare Reproductive Loss Syndrome	Dr. T. Tobin
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An Experiment to Define the Relationship of Prunasin/Mandelonitrile, Hydrogen Cyanide and Certain Bacteria to MRLS and Related Conditions	Dr. L. Harrison
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Study of Bacteria Associated with Mare Reproductive Loss Syndrome	Dr. J. Donahue
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Several projects are also underway in other institutions including reproductive studies at Cornell University, alternative etiological studies including hemlock at Clemson University and field and experimental studies undertaken by two local equine practices in Lexington, Hagyard-Davidson-McGee Associates and Rood and Riddle Equine Hospital. Finance for these projects has been provided from federal funds, the local horse industry, the Grayson Jockey Club Foundation and many private sources.

Communications

In addition to investigating MRLS during 2001 a significant amount of time was spent in providing factual up to date information regarding the syndrome to the equine industry and the media, locally, nationally and internationally. The interest the syndrome generated and the voracious appetite for information proved to be a challenging and at times an almost overwhelming task. Early in May an MRLS web site to address these needs was established by the Department of Veterinary Science in collaboration with the Department of Agricultural Communications at www.uky.edu/Agriculture/VetScience/gluck1.htm. This has proved to be a useful and effective means of communication and by the end of 2001 had been accessed on 33,000 occasions. Not all individuals and groups had immediate access to the Internet and for 2002 a more structured system of communication was established. The Department of Veterinary Science web site continues to be the primary source of information in 2002 and by the end of May had been accessed on 7,500 occasions. MRLS surveillance data provided on a weekly basis includes the total number of accessions to the LDDC and from May 1 the number of confirmed cases of MRLS. Also reported are the number of sick foals, uveitis and pericarditis cases related to MRLS referred to local equine veterinary hospitals plus information from practicing veterinarians on the incidence of EFL in the field. A weekly summary of weather data is also posted and every two weeks the results of the monitoring program undertaken on the sentinel farms. Advice on management strategies to control MRLS has also been provided

A 'list server' was established so that those who requested updates would receive them as they were posted. Several organizations including the Kentucky Association of Equine Practitioners and the Kentucky Thoroughbred Owners and Breeders regularly forward the information on their web site to members. Overall coordination of the flow of information is vested in an Equine

Industry Task Force comprising farm managers and owners representing several horse breeds, practicing equine veterinarians, USDA and state veterinarians and scientists from the University of Kentucky's College of Agriculture. Guidance on the operation of the monitoring program and recommendations for control of MRLS is provided by a small 'oversight group' derived from members of the Task Force.

Economic Impact

The Department of Equine Business at the University of Louisville was commissioned in the summer of 2001 by the Commonwealth of Kentucky, Office of the Governor to quantify the economic loss to the Kentucky equine breeding industry as a result of MRLS. (Thalheimer and Lawrence, 2001, The Economic Loss to the Kentucky Equine Breeding Industry from Mare Reproductive Loss Syndrome (MRLS) of 2001). A survey of 1024 breeders and breeding farm operations involving Thoroughbreds, Standardbreds, Quarter Horses, Paint Horses, Saddlebreds and Tennessee Walking Horses accounting for 96% of all registered foals in the state was undertaken. A response rate of 28% was achieved. It was estimated that 9% of the 2001 foal crop and 26% of the 2002 crop was lost. The total economic loss over a four-year period 2000-2003 was estimated at \$336 million.

Scientific Collaboration

This investigation has since April 2001 involved an enormous number of individuals at the industry, veterinary and scientific level. Each individual in their unique way has contributed to piecing together what has progressively become an increasingly complex puzzle. If and when the final picture is put together that identifies and controls MRLS it will be the result of a concerted and dedicated team effort.

References available upon request.