Survey of Endophyte Infection and Its Associated Toxin in Pastures Grazed by Horses

Brian McCluskey, MS, DVM; Josie Traub-Dargatz, MS, DVM; Lindsay Garber, MS, DVM; and Frank Ross, MS

Neotyphodium coenophialum, an endophytic fungus, and its associated toxin found in tall fescue grass cause health problems in horses grazing in fescue pastures. This study shows endophyte present in multiple geographic regions. Practitioners should be aware of the potential for endophyte-infected pastures and clinical manifestations of ergovaline toxicosis in horses. Authors’ addresses: USDA: APHIS:VS, 755 Parfet St. Ste. 136, Lakewood, CO 80215 (McCluskey); Dept. of Clinical Sciences, Colorado State University, Ft. Collins, CO 80523 (Traub-Dargatz); Centers for Epidemiology and Animal Health, 555 South Howes, Ft. Collins, CO, 80521 (Garber); and National Veterinary Services Laboratories, Ames, IA 50001 (Ross). © 1999 AAEP.

1. Introduction
Tall fescue grass (Festuca arundinacea) is a common forage grass grown in many regions of the United States. In some areas, it is the dominant grass species and is considered adaptive and aggressive. In many instances in the Midwest and South, fescue has replaced established forages. Neotyphodium coenophialum, formerly known as Acremonium coenophialum, is an endophytic fungus that commonly infects tall fescue grass. Endophyte-infected fescue grass is more productive and resistant to drought and insect predation than fescue grass free of endophyte and thus has competitive advantage. The nutritional value of tall fescue grass was found to be adequate in most components. Geldings fed chopped alfalfa, tall fescue or caucasian blue stem hay showed no differences in body weight over the 15-day feeding trial. Tall fescue’s value as a forage for horses has been questioned only because of its susceptibility to infection with endophytic fungi and the potential of the endophyte to produce toxins harmful to horses.

Production of ergopeptine alkaloids, primarily ergovaline, by endophytes causes reproductive and other disorders in horses grazed on endophyte-infected fescue. Although ergovaline is the primary ergot alkaloid found in N. coenophialum-infected fescue, other ergot alkaloids may be present in equal concentrations as the ergovaline. The total concentrations of ergot alkaloids found in endophyte-infected fescue varies seasonally, although variations in the concentration of individual alkaloids have not been examined.

Gravid mares grazing endophyte-infected fescue either continuously or from day 300 of gestation to parturition had increased gestation lengths. Mares may carry foals for 370 days or longer when consuming endophyte-infected fescue. This extended gestation period may also result in dystocia, stillborn foals, retained placentas and in some cases mare...
Pasture samples were forwarded to the National Animal Health Monitoring System's (NAHMS) Equine '98 Study was designed to provide information on the nation's equine population for education and research purposes. NAHMS is sponsored by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. One objective of the Equine '98 Study was to investigate the prevalence of endophyte infected pasture among participating operations and to measure the levels of ergovaline in infected pasture samples. Associations by geographic region and other factors were examined.

2. Materials and Methods
The USDA collected data on equine health and management practices through four personal interviews with a representative sample of equine operations in 28 states (Alabama, California, Colorado, Florida, Georgia, Illinois, Indiana, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Missouri, Montana, New Jersey, New Mexico, New York, Ohio, Oklahoma, Oregon, Pennsylvania, Tennessee, Texas, Virginia, Washington, Wisconsin, Wyoming). A list frame representing nonfarm commercial operations and an area frame based on a land use stratification, including agricultural-urban areas, were combined to create the sampling frame. The reference population for the first phase of the Equine '98 Study, which focused on baseline information for the equine industry, included all operations with one or more equids in the 28 states. The reference population for the second phase of the study included operations with three or more horses present on January 1, 1998, in the 28 states, excluding race tracks.

During summer visits to study premises, participants were questioned about the availability of pasture to resident equids. If pasture was available, participants were asked if a sample could be obtained and tested for the presence of endophyte and ergovaline. Pasture sampling was not limited to fescue grass pasture. Participants were also asked to describe the primary forage type in the pasture. Estimates of percent edible vegetation and forage quality were made at the time of sample collection by the USDA veterinarian conducting the interview. Pasture samples were collected by cutting forage as close to the ground as possible from 20 locations in the pasture being grazed by most of the horses at the time of the visit. Samples were collected between June 15 and September 11, 1998. Pasture samples were forwarded to the National Veterinary Services Laboratories for endophyte and ergovaline testing.

Laboratory testing procedures included the following: Samples were initially screened for the presence of endophyte. Approximately 0.25-in cross-sections were cut from 20 plant stems (tillers) from pasture samples and tested for the presence of endophyte using the Agrinostics Phytoscreen Kit. Tillers were placed on a buffer-soaked membrane and incubated overnight at 4°C. The membrane was blotted dry and then incubated at room temperature with anti-Neotyphodium coenophialum antibody. After rinsing, the membrane was incubated with a chromogenic solution to see the presence (pink color) or absence (no color) of endophyte. Ergovaline values were determined in pasture samples that tested positive for endophytes by the screening test. Samples were freeze-dried and ground in a hammer mill to pass through a 2-mm screen. A portion of the ground sample was extracted in methanol. The methanol solution was filtered through filter paper and then passed through a solid-phase extraction (SPE) cartridge for cleanup. The resulting extract was then injected onto an high-performance liquid chromatograph (HPLC) equipped with a fluorescence detector and a reversed-phase column. Ergovaline concentrations, in parts per billion (ppb), were based on comparison of detector response to a known standard.

For computation of point estimates, weights representing the inverse of the probability of selection for each horse operation adjusted for nonresponse were used. Statistical software (SUDAAN) that incorporates the study design stratification (by region and operation size) variance estimates was used to estimate variances associated with the weighted point estimates. The chi-square test for independence was applied to selected variables, and p < 0.05 was considered significant.

3. Results
Overall, 894 of 1082 (83%) participants eligible for sampling agreed to have pasture samples collected and tested. A total of 564 (63%) of the pastures were test positive for endophyte and 301 (34%) were test positive for toxin. A pasture sample was considered positive if any of the 20 tillers tested were found to contain endophyte. Ergovaline levels ranged from 0 to 1622 ppb, and 75% of samples had ergovaline levels between 0 and 24 ppb. Regional estimates and standard errors of endophyte and toxin prevalence are presented in Fig. 1. There was no statistical difference among regions for the percentage of pastures with endophyte (p = 0.24) or toxin (p = 0.19). The presence of endophyte in pastures with and without fescue, as determined by the participant, was 79% and 57% (SE 4.62), respectively (p = 0.01). The presence of toxin in samples with and without fescue was 54% and 21% (SE 7.4), respectively (p = 0.004). The percentages of samples positive for endophyte and toxin were not...
statistically different based on the estimated percentage of edible vegetation or the estimated forage quality in the summer of 1998.

4. Discussion
Endophyte was identified on more than half of operations participating in all four regions, indicating that endophyte-infected pastures are located on horse operations in all geographic areas of the United States. These results are similar to those of a previous survey in 26 states, which found that 58% of plant samples were positive for endophyte.\(^9\) The commercial test kit was used to screen pasture samples for endophyte. Field-based test sensitivity and specificity data were not available for this kit, resulting in a potential for overestimation or underestimation of the percentage of operations with endophyte in pastures. Most pastures that had endophyte had less than 50% of the tillers test positive for endophyte; however, low initial infection rates will increase over time without pasture management intervention because of infected fescue's ability to compete with noninfected fescue. The HPLC assay is capable of detecting ergovaline at very low levels, providing a high degree of confidence in positive toxin results. Approximately one-third of operations participating in the Northeastern, Southern, and Central regions had pastures with detectable levels of ergovaline while 18% of those operations participating from the Western region were positive for ergovaline. Rainfall, season and other environmental factors have been associated with variations in toxin levels. In this study pasture samples were taken only once during the summer months. Sequential sampling of the same pasture may have shown different ergovaline levels although any detectable level of toxin may be harmful to horses.\(^c\)

Participants reported whether the pastures to be sampled contained fescue grass or not. No tests were conducted to confirm or refute this information. The presence of endophyte in 57% of samples and toxin in 21% of samples reported to not contain fescue grass could be explained by endophyte infecting other grasses or by participants incorrectly identifying pastures as not containing fescue grass. There was no association of endophyte infection or the presence of toxin with either the interviewer-estimated percentage of edible vegetation or the estimated forage quality. Fescue's aggressive and adaptive nature may allow it to be the predominant grass type in overgrazed pastures.\(^1\)

Historically, strategies to lower the risk of health problems associated with horses grazing in fescue grass pastures have been successful. Elimination of existing infected fescue through herbicide application or with tillage is one method of reducing exposure to infected pastures. After removal of infected plants, the pasture can be reseeded with certified...
fungus-free fescue or other forage grasses. For many farm managers, reseeding is not economical; therefore, pregnant mares should be removed from infected pastures 30 to 45 days before foaling. Domperidone, a dopamine antagonist, may also be used to treat mares that must graze in infected fescue pastures. Domperidone administration should be initiated 30 days before foaling at a dose of 1.1 mg/kg PO once per day.

Farm management practices regarding what percentage of the participants' horses' diets the pasture sampled provided, whether pregnant mares were removed from fescue grass pastures during pregnancy, or whether domperidone was administered to pregnant mares were not determined in this study.

Identification of endophyte-infected pasture and its associated toxin in all geographic regions surveyed emphasizes the widespread nature of this potential problem. Practitioners should be familiar with signs associated with fescue toxicity and with appropriate prevention and treatment strategies to use if endophyte-infected fescue is identified in their clients' pastures.

References

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