Case Report: Investigating an outbreak of tremorgenic mycotoxicosis in beef cows on pasture in Mississippi due to ergot (*Claviceps paspali*) infection in dallisgrass (*Paspalum dilatatum*)

***W. Isaac Jumper,**[1] DVM, PhD, Dipl. ACVPM; **Clare C. Brown,**[1] DVM; **Stephen T. Lee,**[2] PhD; **Daniel Cook,**[2] PhD; **Justin M. Stilwell,**[1] DVM, PhD, Dipl. ACVP; **Kelsey M. Harvey,**[3] PhD

[1] College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762
[2] United State Department of Agriculture, Agriculture Research Service, Poisonous Plant Research Laboratory, Logan, UT 84341
[3] Prairie Research Unit, Mississippi State University, Prairie, MS 39756
***Corresponding author:** Dr. W. Isaac Jumper, **isaac.jumper@msstate.edu**

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Abstract

The Mississippi State University College of Veterinary Medicine Ambulatory Service examined a 3-year-old Charolais-cross cow on October 19, 2023, for lateral recumbency, inability to rise and generalized tremors. Within 4 hours following initial case discovery, 20 additional cases were discovered in 2 adjacent pastures, including one mortality. Clinical signs ranged in severity from mild tremors to severe ataxia, hyperexcitability, aggression, lateral recumbency and death. All affected cows had calved in the spring of 2023, and all 2023 spring calves were weaned 3 days prior on October 16. Overnight on October 16-17, one group of cows pushed down a fence, and 32 cows entered a pasture adjacent to their weaned calves. Following discovery of cases on October 19, all cattle were removed from the affected pasture and grass hay was provided immediately. Necropsy of 2 affected animals as well as serum chemistries of 5 affected cows revealed non-specific findings. However, abundant dallisgrass (*Paspalum dilatatum*) with obvious *Claviceps paspali* infection was present on the pasture that the cattle entered overnight on October 16-17. High-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis of samples collected from *C. paspali* infected pasture as well as rumen and abomasum contents collected at necropsy revealed several tremorgenic indole diterpene alkaloids. Additionally, DNA metabarcoding of rumen contents and forage samples confirmed the ingestion of dallisgrass infected with *C. paspali*. Upon recheck on October 20, all previously affected cows appeared to be recovering, and complete resolution of clinical signs had occurred by October 23.

Introduction

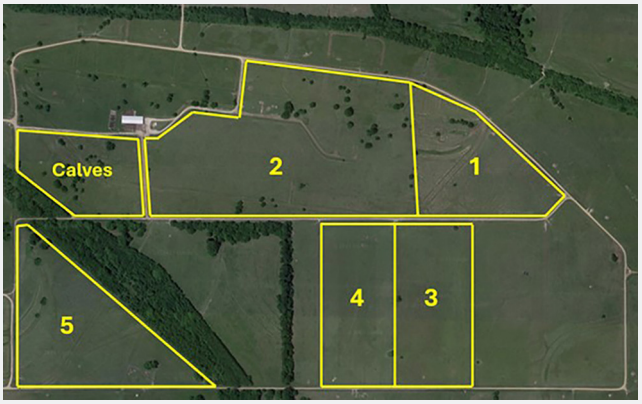
Dallisgrass (*Paspalum dilatatum*) is a warm-season, perennial forage commonly found in pastures across Mississippi and other southeastern states.[1] Native to South America, dallisgrass is well adapted to clay or loam soils and often initiates growth earlier than other warm-season perennial grasses and maintains growth later into the fall.[2] Dallisgrass seedheads are susceptible to ergot (*Claviceps paspali*) infection during development. The fungus produces a dark brown to black ergot fungal body, or sclerotium, that is grossly visible in the plant's seedhead on pasture. The ergot body produces copious fungal spores, as well as a sticky, sweet exudate (i.e., honeydew) that sticks to the legs of animals and insects, allowing the fungal spores to be transported to other dallisgrass stands.[3] Numerous tremorgenic indole-diterpenoid alkaloids such as paspalinine, paspalitrem A and paspalitrem B have been isolated from the ergot body produced by *C. paspali*.[3] Ingestion of either the ergot body or the honeydew predisposes livestock to the development of dallisgrass staggers characterized by tremors of the head, neck and flank, uncoordinated movement, hyperexcitability, ataxia, seizures and collapse.[3-7] Outbreaks of dallisgrass staggers in beef cattle on pasture often begin within 2 to 3 days of ingesting contaminated forage, typically displaying high morbidity and low mortality.[8] The growth and life cycle of *C. paspali* in Mississippi pastures is well-described and has long been associated with sporadic outbreaks of neurologic disease in grazing livestock.[4] The following report describes an outbreak of tremors, ataxia and hyperexcitability attributed to *C. paspali* mycotoxicosis in beef cows on pasture in Mississippi.

Case description

Herd history

The affected herd consisted of approximately 350 Angus-Charolais influenced commercial cows that were split between spring (i.e., February-April) and fall (i.e., August-November) calving seasons. All affected individuals were members of the spring calving herd (n = 147 cows). On October 16, 3 days prior to the initial case, calves born between February and April of 2023 had been separated from these cows. No other health interventions (e.g., vaccines, dewormer, pregnancy examinations, etc.) were performed on the cows at this time. After weaning, all cows were divided into 5 pasture groups of approximately 35 to 37 cows each and housed in separate pastures approximately 25 acres in size. Figure 1 displays the location of weaned calves relative to pastures of interest in this outbreak. One of these groups of cows (n = 32) was moved to pasture 1 (Figure 1) following separation from their calves. During the night of October 16 and early morning of October

Figure 1: Satellite map image of the property where the outbreak took place showing the relative locations of pastures where cows and calves were located. Note that dallisgrass seedheads were found in pasture 2, while all cases were found in pastures 3 and 4 between 2-3 days following transfer from pasture 2. Cattle were moved to pasture 5 shortly after the outbreak was discovered.



17, the cows in pasture 1 escaped into pasture 2 to get back to their calves. These cows were discovered on the morning of October 17 and removed from pasture 2 and placed in pastures 3 and 4 over the next 24 to 36 hours (Figure 1).

A granular, trace mineral supplement was provided free choice to cattle in all pastures; however, no other hay or supplements were being offered. All pastures consisted of mixed perennial grasses including tall fescue (*Lolium arundinaceum*) as well as bahiagrass (*Paspalum notatum* Flugge), dallisgrass (*Paspalum dilatatum*), and common bermudagrass (*Cynodon dactylon*). Water was available in all pastures through automatic, ball-valve waterers supplied by a well. Pastures 1, 3 and 4 had been mowed by operation management approximately 2 weeks prior to placement of cows in them on October 16; however, pasture 2 had not been grazed or mowed in the previous 3 months. No fertilizers, herbicides or insecticides had been applied to pastures 1, 2, 3 or 4 in the previous 6 months. No females had been introduced into this herd in the previous 2 years; however, 1 bull was purchased in November 2022, and another in February 2023. In both cases, bulls were held in quarantine for 28 days prior to breeding season. The operation has no fence line contact with outside or neighboring cattle.

Initial case physical exam findings

On the afternoon of October 19, 2023, the Mississippi State University College of Veterinary Medicine Large Animal Ambulatory Service was contacted by employees of the previously described beef cow-calf herd to evaluate a 2-year-old Charolais-cross cow that was down in pasture 4 (Figure 1) and unable to rise. The cow, J098, was in right lateral recumbency at the time of veterinarian arrival, and generalized muscle fasciculations and tremors were present. Limited physical examination of J098 revealed a rectal temperature of 102.2 °F (39 °C), pulse of 92 beats per minute, and a respiratory rate of 28 breaths per minute. No abnormalities were auscultated in the left lung field or the heart from the left thorax. The left prescapular and prefemoral lymph nodes, as well as submandibular and parotid lymph nodes, palpated normally. The right thorax could not be assessed well due to the cow's position in right lateral recumbency. The cow was estimated to weigh approximately 1,200 lbs (544 kg), and her mucous membranes as evaluated from the vulva were pink and moist, with a capillary refill time of 2 seconds. Initially, the cow's mentation was depressed with little effort exerted to correct her lateral recumbency when attempts were made to manually position the cow in sternal recumbency. However, following initial steps in the physical examination and multiple attempts to manually position the cow in sternal recumbency, J098 became agitated and displayed frantic behavior while attempting to stand. Rectal palpation and abdominal auscultation were precluded due to the cow's hyperexcitability. Muscle fasciculations and tremors were exacerbated when the cow was approached or examined and were particularly noticeable in the musculature of the proximal forelimb, trunk and proximal hindlimb. The tremors grew more prominent as the cow struggled to stand. After several failed attempts, J098 was able to stand, but displayed severe ataxia. After several steps, she fell and resumed right lateral recumbency. No evidence of circling or a head tilt were observed during the time the cow was able to stand; however, decreased proprioception was evident in the form of stumbling, staggering and loss of resistance to lateral falls (i.e., loss of antigravity myotatic reflex).

Initial case treatment

To facilitate treatment, the cow was rolled into sternal recumbency, a halter was placed, the head was pulled to the right side, and the end of the halter tied to the right tarsus. Blood was collected from the jugular vein in a lithium heparin-containing tube for blood chemistry analysis[a]. Treatment included 500 mL calcium-magnesium-phosphorus-potassium (CMPK) administered intravenously (IV) into the jugular vein, 500 mL of calcium gluconate subcutaneously, florfenicol at 40 mg/kg subcutaneously in the neck, thiamine HCl at 10 mg/kg with half of the dose given IV in the jugular vein, and half of the dose given intramuscularly in the neck, and 20 mg of dexamethasone given intramuscularly in the neck. Following treatment, the halter was removed, and the cow struggled to rise and stand. Previously observed ataxia, muscle fasciculations, and tremors persisted following treatment.

Additional cases

Further investigation of pastures 3 and 4 throughout the evening of October 19 and the morning of October 20 revealed 20 additional cases with similar clinical signs of varying severity, for a total of 21 clinically affected cows out of the 32 total cows (65%) placed on pastures 3 and 4. Clinical signs in these cattle ranged from lateral recumbency and inability to rise, to severe ataxia and tremors, to mild tremors that did not hinder the ability of the animal to walk and graze. Affected cows ranged from 2 to 14 years of age, and one 3-year-old cow identified as J065 was found dead in pasture 4 on the afternoon of October 19. The most severely affected cattle (i.e., those down and unable to rise) were treated similarly to the initial case, however, some severely affected cows in pasture 4 were not treated due to marked hyperexcitability, aggression and clinician concerns that the stress from restraining these cows in the pasture would lead to death. On the evening of October 19, all animals in pastures 3 and 4 that could ambulate well enough to be moved were removed from these pastures and placed together in a new pasture identified as pasture 5 (Figure 1). Pasture 5 had been extensively grazed previously, resulting in very little forage availability, therefore, hay was provided to these cows ad libitum.

Clinical outcomes

A total of 5 cows, including J098, could not be removed from pastures 3 and 4 on the evening of October 19 because they were unable to rise and ambulate well enough to move out of the two pastures. On the morning of October 20, 4 of these cows were standing and clinical signs were significantly improved. Three of the 4 cows had not been treated the previous evening. Only 1 cow, a 5-year-old cow identified as F039, remained down on October 20. Due to persistence of severe clinical signs, F039 was euthanized and submitted for diagnostic necropsy. Over the next 72 hours, clinical signs continued to improve in all other affected animals, both treated and untreated. Examination of cattle on October 23 showed complete resolution of all clinical signs.

Results

Two affected cows from the herd, J065 and F039, were submitted to the Mississippi State University College of Veterinary Medicine Diagnostic Lab for postmortem examination. The first, an approximately 3-year-old Angus-cross cow (J065), was examined within 3 hours of being found dead on October 19, 2023. A second affected cow, an approximately 6-year-old Charolais-cross (F039), was euthanized the following day, October 20, 2023, and examined within 2 hours following death.

Gross necropsy findings

Relevant postmortem findings in both cows were similar and thus described together unless otherwise indicated. Both animals were in fair to good body condition (BCS 4 to 5/9) and exhibited largely non-specific findings including mild (F039) to moderate (J065) pulmonary edema and pulmonary petechiae (J065), epicardial hemorrhages, and endocardial and laryngeal hemorrhages (J065). The forestomachs of both cows contained abundant moist, short-stemmed, green forage material. The abomasal contents were similar, with the addition of gravel and numerous tan-to-dark brown to black seedheads (Figure 2). The abomasal and jejunal mucosa of F039 was mildly to moderately reddened, suggestive for abomasitis and enteritis. The small intestines contained liquid green material which transitioned to soft green-brown feces in the cecum and colon. F039 had black, arborizing tracts within the liver, consistent with liver fluke tracts. No other significant gross lesions were observed in the remainder of examined organs.

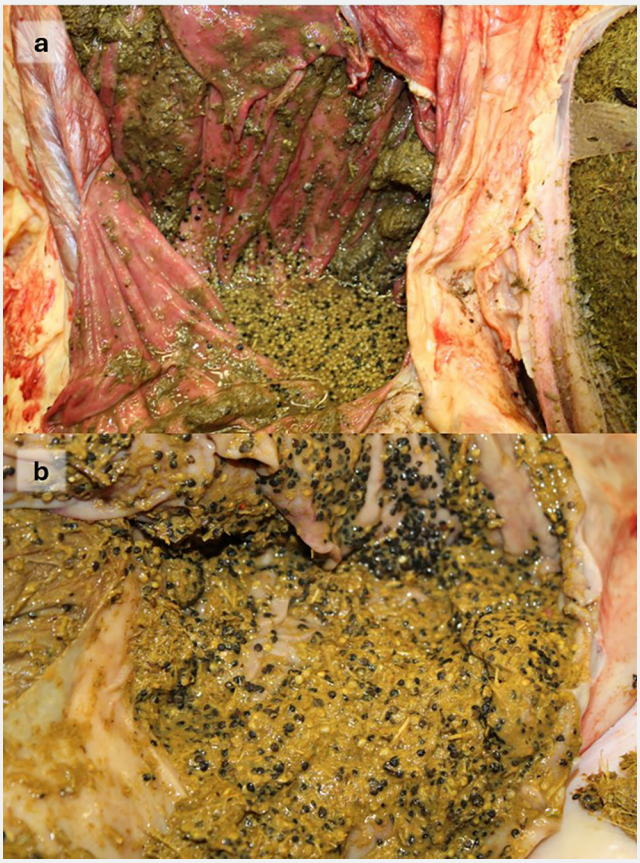
Histopathology findings

Similar to the gross examination, microscopic lesions were largely nonspecific and of limited severity. Identified lesions included scant to mild cerebral edema (J065), gliosis, pulmonary edema, myocarditis (J065), chronic hepatic fluke migration with fibrosis (F039), and eosinophilic abomasitis and enteritis (F039).

Serum chemistry and other diagnostic test result

Blood samples were collected from a total of 5 affected cattle, and serum chemistry results for each animal are listed in Table 1. Samples were collected from cows J098 and H112 at the time of treatment on October 19. Blood was collected from J098 for serum chemistry only at the time of treatment on October 20. Nitrate testing results on ocular fluid from J065 and serum from J098 and H112 were negative. Ammonia test results on serum samples from J098 and H112 were also negative. A section of frontal lobe from each necropsy (J065 and F039) was submitted for sodium analysis to investigate potential salt toxicity[b]. Brain tissue from J065 and F039 contained sodium concentrations of 2,154 ppm and 1,875 ppm (reference range: < 2,000 ppm), respectively.

Figure 2: Contents of the abomasum of a) cow F039 and b) cow J065 demonstrating an abundance of seedheads within the gastrointestinal tract of each cow.



Forage sample collection and preparation

The predominant forage present in pasture 2 was dallisgrass, with abundant seedhead development and evidence of ergot growth across the pasture. Little to no dallisgrass seedheads were found in pastures 1, 3, 4 or 5 due to the pastures being previously grazed and/or mowed. Samples of affected dallisgrass were collected by hand from across pasture 2, frozen and shipped to the United States Department of Agriculture (USDA) Agriculture Research Service (ARS) Poisonous Plant Research Laboratory in Logan, Utah for further analysis. Upon arrival to the lab, plant material was air dried (30 °C), ground and weighed (50 mg) into 2.0 mL screw cap microcentrifuge tubes and extracted with 1 mL isopropyl alcohol by mechanical rotation for 3 hours. Rumen and abomasum samples were freeze dried, ground and weighed (1.0 g rumen, 0.50 g abomasum) into 20 mL vials. The rumen (1.0 g) and abomasum (0.50 g) samples were extracted with 10- and 5-mL isopropyl alcohol, respectively, by mechanical rotation for 3 hours. The samples were centrifuged (10 min), and an aliquot (150 µL) of the supernatant was transferred to 300 µL autosample vials for high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis.

Table 1: Serum clinical chemistry values and reference intervals. Cholesterol and phosphorus were not available on the afterhours chemistry panel when the samples were processed. Abbreviations below are as follows: ALP – alkaline phosphatase, AST – aspartate aminotransferase, BUN – blood urea nitrogen, CK – creatine kinase, GGT – gamma-glutamyltransferase.

Serum biochemistry value	Patient identification					Reference values
	J065*†	J098†	H112†	F039‡	9098‡	
Albumin	0.8	2.7	3.2	3.0	2.3	2.4-3.7
ALP	65.0	99.0	58.0	43	33	25-160
AST	138.0	285.0	172.0	114	95	64-76
Total Bilirubin	<0.1	0.3	0.4	0.5	0.5	0.2-0.7
BUN	9.0	8.0	3.0	10	12	6-25
Calcium (Ca)	7.4	9.3	9.2	9.3	9.4	7.8-10.5
Chloride (Cl)	111.0	105.0	102.0	102.0	95.8	97-111
Cholesterol	N/A	N/A	N/A	142	145	78-142
CK	1120.0	8760.0	264.0	2802	736	64-405
Creatinine	1.47	1.8	1.20	1.44	1.28	1-2.1
GGT	5.0	31.0	26.0	17	18	10-35
Globulin	N/A	4.5	5.3	4.8	6.8	2.5-4.4
Glucose	5.0	108.0	111.0	163	100	61-102
Magnesium (Mg)	3.49	2.41	1.93	1.8	1.6	2.0-2.8
Phosphorus (P)	N/A	N/A	N/A	3.4	9.1	4.0-7.1
Potassium (K)	11.8	4.4	3.6	4.08	4.69	3.9-5.8
Sodium (Na)	136.2	146	147.0	145.2	141.1	132-152
Total protein	0.8	7.2	8.5	7.8	9.1	7.0-8.9

* = Chemistry panel performed on ocular fluid collected at postmortem examination, reference ranges do not apply.

† = Values obtained by: IDEXX Catalyst One Chemistry Analyzer, IDEXX Laboratories, Inc., Westbrook, ME 04092

‡ = Values obtained by: Alfa Wasserman Vet Axcel Clinical Chemistry Analyzer, Alfa Wasserman Diagnostic Technologies, LLC., West Caldwell, NJ 07006

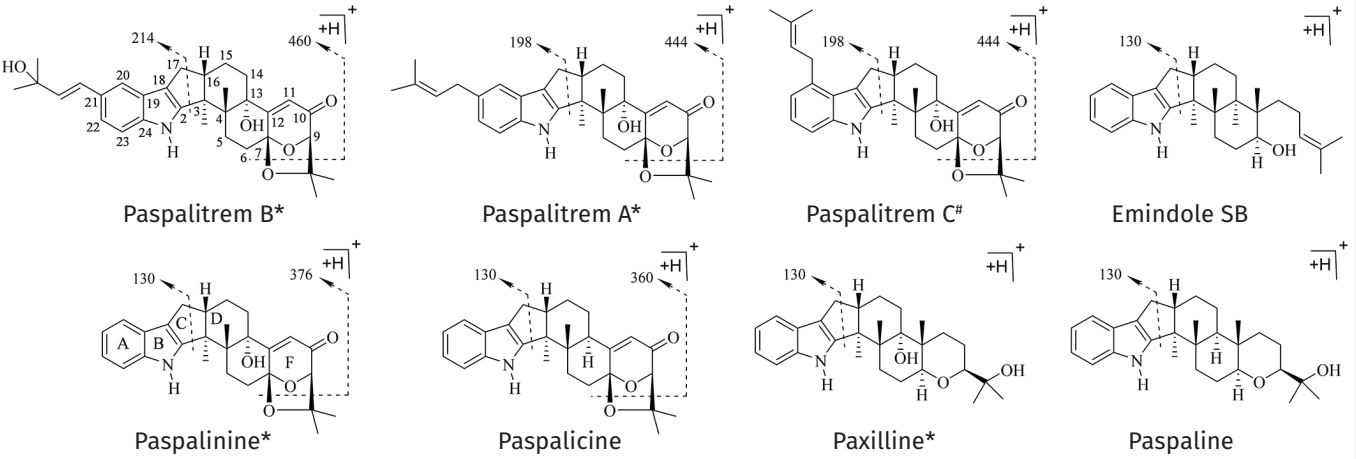
Indole diterpene alkaloid analysis methods and results

Samples (10 µL) were injected onto a 100 x 2.1 mm i.d., 5 µm, Betasil™ C18 with a 10 x 2.1 mm i.d. guard column of the same material[c]. The samples were eluted from the column with a gradient flow at a flow rate of 0.300 mL/min. The mobile phase program was 0.1 % formic acid/acetonitrile (90:10, v/v) for 1 min followed by a linear gradient to a composition of 100% acetonitrile at 40 min.

For high resolution mass spectrometric analysis of high-performance liquid chromatography (HPLC-HRMS) peaks, the mobile phase was delivered and samples injected using an UltiMate™ 3000 HPLC[d] and the column eluent was connected to the heated electrospray source of an Exactive™ Plus Orbitrap™ high resolution mass spectrometer[d] calibrated as per the manufacturer's instructions and with a scan range 100 to 800 Da, resolution 70,000, microscans 1, sheath gas flow 35, auxiliary gas flow 10, spray voltage 4 kV, capillary temperature 320 °C, S lens RF field 55, and auxiliary gas temperature 300 °C operated in the positive ion mode. Chromatographic peaks were identified by generating reconstructed HPLC-HRMS selected ion chromatograms from the calculated MH^+ masses of the compounds of interest to 5 decimals places and with a mass tolerance of 10 ppm. Under these conditions, indole diterpene alkaloids identified as paspalitrem B, paxilline, paspalinine, paspalicine, paspalitrem A, paspalitrem C, paspaline and emindole SB eluted at 22.9, 25.1, 26.6, 30.3, 32.2, 33.1, 35.2 and 36.2 min, respectively (Figure 3, 4; Table 2). High performance liquid chromatography-higher energy collisional dissociation-tandem mass spectroscopy (HPLC-HCD-MS/MS) data were acquired for paspalitrem B (MH^+, m/z 518.29), paxilline, (MH^+, m/z 436.25), paspalinine (MH^+, m/z 434.23), paspalicine (MH^+, m/z 418.24) (paspalitrem A (MH^+, m/z 502.30) and paspalitrem C (MH^+, m/z 502.30), paspaline (MH^+, m/z 422.31) and emindol SB (MH^+, m/z 406.31), these masses were fragmented under HCD conditions with an isolation width (m/z) 2.0 and normalized collision energy 25.0.

Indole diterpene alkaloids were not detected in the extract from the seedheads that did not appear to be ergotized (Figure 5). However, eight HPLC-HRMS peaks consistent with paspalitrem B, paxilline, paspalinine, paspalicine (tentative), paspalitrem A, paspalitrem C (tentative), paspaline and emindole SB and with observed MH^+ within 6 ppm of the calculated masses were identified in the isopropyl extract of the seedheads that appeared to be infected with ergot (Figures 3, 4 and 5; Table 2).

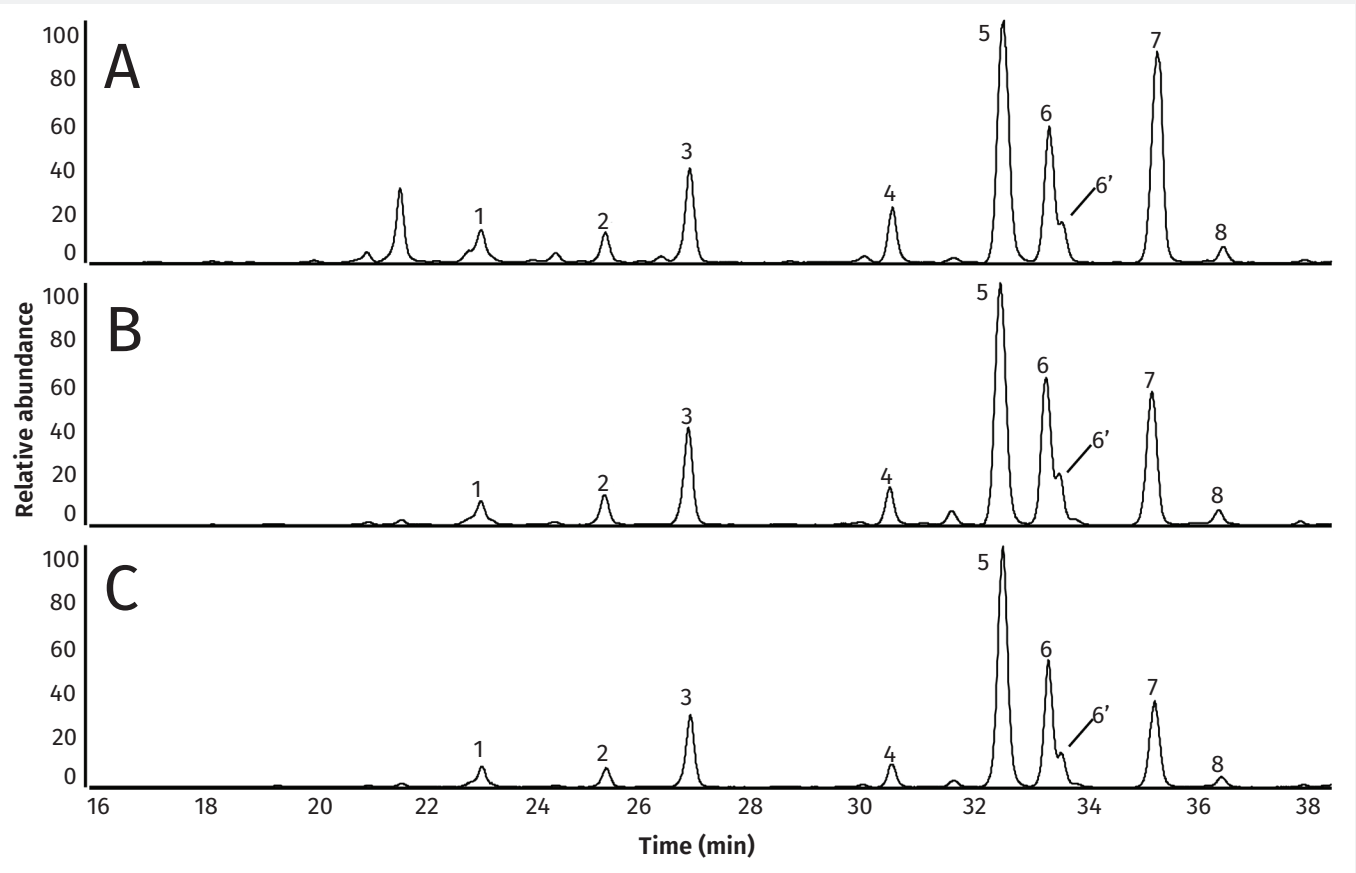
Figure 3: Chemical structures of paspalitrem B, paspalinine, paspalitrem A, paspalitrem C, paspaline and emindole SB with plausible MS2 fragments.



* Indicates tremorgenic indole diterpene alkaloids.

\# Paspalitrem C is hypothesized to be tremorgenic based on the presence of tertiary hydroxy group at C-13.

Figure 4: Extracted HPLC-HRMS ion chromatograms (m/z = 518.28708, 436.24587, 434.23185, 418.23628, 502.29469, 502.29478, 422.30423, 406.30880) from isopropyl alcohol extracts of: A) fungal infected dallisgrass seedheads; B) rumen contents from a cow suspected of grazing dallisgrass and C) abomasum contents from a cow suspected of grazing dallisgrass.



Peak m/z identifications: 1 (MH+ = 518.28708, paspalitrem B); 2 (MH+ = 436.24587, paxilline); 3 (MH+ = 434.23184, paspalinine); 4 (MH+ = 418.23628, paspalicine); 5 (MH+ = 502.29469, paspalitrem A); 6 (MH+ = 502.29478, paspalitrem C or possible paspalitrem C isomer); 6 (MH+ = 502.29478, possible paspalitrem C isomer or paspalitrem C); 7 (MH+ = 422.30423, paspaline); 8 (MH+ = 406.30880, emindole SB).

Table 2: HPLC and mass spectrometry data for indole diterpene alkaloids in isopropyl alcohol extracts of fungal infected dallisgrass seedheads, rumen contents and abomasum contents.

Identity*	RT min	MH+ (*m/z*)	Calc mol formula (MH+)	D (ppm)	HCD MS/MS (m/z) (% Rel abundance)
Paspalitrem B, 1	22.9	518.28708	C32H40NO5	-5.827	518 (20), 503 (28), 502 (34), 460 (24), 443 (26), 214 (100), 196 (95), 168 (78), 156 (38)
Paxilline, 2	25.1	436.24587	C27H34NO4	-5.421	436 (3), 420 (10), 378 (7), 360 (34), 347 (21), 182 (20), 130 (84)
Paspalinine, 3	26.6	434.23184	C27H32NO4	-1.716	434 (6), 376 (40), 130 (100)
Paspalicine, 4 †	30.3	418.23628	C27H32NO3	-3.324	418 (4), 360 (12), 131 (7), 130 (100)
Paspalitrem A, 5	32.2	502.29469	C32H40NO4	-0.986	502 (1), 444 (24), 198 (100)
Paspalitrem C, 6 †	33.1	502.29478	C32H40NO4	-0.807	502 (1), 444 (26), 198 (100)
Paspaline, 7	35.0	422.30423	C28H40NO2	-2.666	422 (7), 131 (7), 130 (100)
Emindole SB, 8	36.2	406.30880	C28H40NO	-4.040	406 (13), 388 (10), 257 (9), 182 (7), 131 (7), 130 (100)

* = Identities confirmed by comparison high resolution mass spectral data with calculated masses and comparison of HPLC retention times and MS/MS retention data of Uhlig et al.[9,10]

† = Tentative identifications by Uhlig et al.[9,10]

Figure 5: Ergotized (*Claviceps paspali*) (left) and non-ergotized (right) dallisgrass (*Paspalum dilatatum* seedheads collected from pasture 2 grazed by poisoned cows.

This extract was further analyzed by HPLC-MS/MS. A major MS/MS fragment of the protonated indole diterpene alkaloids of m/z 214, 130, 130, 130, 198, 198, 130 and 130 is consistent with fragmentation near the indole portion of the alkaloids through the C-ring of paspalitrem B, paxilline, paspalinine, paspalicine (tentative), paspalitrem A, paspalitrem C (tentative), paspaline, and emindole SB, respectively (Figure 3, Table 2).[9,10] Another major fragment of m/z 460, 360, 376, 360, 444, and 444 observed for paspalitrem B, paxilline, paspalinine, paspalicine (tentative), paspalitrem A, and paspalitrem C (tentative), respectively, is a loss of 58 amu, presumably due to loss of acetone, $(CH_3)_2CO$, from fragmentation of the alkaloids through the 7,27-oxido linkage (Figure 3, Table 2).[9,10] These 8 indole diterpene alkaloids and major fragments were previously reported in ergotized dallisgrass seedheads.[9] Paspalitrem B, paxilline, paspalinine, paspalitrem A and paspaline have been confirmed in ergotized dallisgrass seedheads using authenticated standards of these alkaloids.[9] In contrast, the lack of authenticated standards, but convincing high-resolution mass data and MS/MS fragmentation for paspalicine and paspalitrem C, resulted in tentative identifications of these compounds.[9,10] Alkaloids identified as 6 and 6' herein, have similar HPLC retention times, have essentially the same mass, and similar MS/MS fragmentation as paspalitrem A. Both 6 and 6' likely possess a C_5H_9 side chain off the C-ring like paspalitrem A, but at a different position on the C-ring to paspalitrem A and to each other. It is likely that one of these alkaloids is paspalitrem C and the other being a structural isomer of paspalitrem C.[9,10]

Isopropyl alcohol extracts from the rumen content and abomasum content of two cows (J065 and F039) with clinical signs of poisoning and that died were also analyzed by HPLC-HRMS. The same 8 indole diterpene alkaloids were observed in the rumen and abomasum contents from both animals as were observed in the extract from ergot infected seedheads, confirming that the poisoned cows ingested the infected dallisgrass (Figure 4, 2 and 5).

Four of the indole diterpene alkaloids (paspalitrem B, paxilline, paspalinine and paspalitrem) observed in the extract from the ergot infected seedheads, rumen contents and abomasum possess a tertiary hydroxy group at C-13 and are known tremorgens.[3] In addition, paspalitrem C is hypothesized to be tremorgenic based on the presence of tertiary hydroxy group at C-13 (Figure 3).[11]

DNA metabarcoding analysis methods and result

A subsample of the rumen contents from each respective sample was sent to a commercial laboratory for DNA metabarcoding analysis[e]. DNA was extracted from the rumen contents and forage sample using the Omega Biotek Mag-Bind® Universal Pathogen Core Kit (4x96 Preps) (Cat. No. / ID: M4030-01) according to the manufacturer's protocol. DNA primers, trnL c and h were used for PCR amplification of the rumen contents.

DNA primers ITS1-F and ITS-2 were used for PCR amplification of the forage sample. Both forward and reverse primers also contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Amplicons were cleaned and a second round of PCR was performed to complete the sequencing library construct, appending the final Illumina sequencing adapters and integrating sample-specific, dual index sequences (2 x 10 bp). Final indexed amplicons from each sample were cleaned and normalized using mag-bind normalization. Sample library pools were sent for sequencing on an Illumina MiSeq™ (San Diego, CA). Raw sequence data were demultiplexed using pheniqs v2.1.0 enforcing strict matching of sample barcode indices (i.e., no errors).[12] For each sample, reads were then clustered using the unoise3 denoising algorithm as implemented in vsearch, using an alpha value of 5 and discarding unique raw sequences observed less than 8 times. Counts of the resulting exact sequence variants (ESVs) were then compiled.[13] For each final ESV, a consensus taxonomy was assigned using a custom best-hits algorithm and a reference database consisting of publicly available GenBank sequences as well as Jonah Ventures voucher sequence records. The consensus taxonomy was then generated using either all 100% matching reference sequences or all reference sequences within 1% of the top match. The relative abundance of each ESV for a respective sample was calculated by dividing the number of reads for a respective ESV by the total number of reads from that respective sample. ESVs calculated to be greater than 2% from any one sample were considered for further analyses. The complete data set of the DNA metabarcoding results are available upon request from the authors.

Forage sample DNA metabarcoding confirmed the presence of dallisgrass and another ESV of an unknown Poaceae. Rumen DNA metabarcoding identified dalligrass in the rumen contents of both animals. Dallisgrass represented 94 and 6% of the total reads in each respective sample. Other ESVs that corresponded to greater than 2% of the total reads in any one of the rumen samples corresponded to the following plant families/genera: Poaceae (grass, 3 ESVs); Rubiaceae, *Spermacoce* (buttonweed, 2 ESVs); Euphorbiaceae, *Euphorbia*, (spurge, 1 ESV); and Polygonacaeae, *Polygonum* (smartweed/knotweed, 1 ESV). DNA metabarcoding of the forage sample identified several fungi, one of which was *Claviceps paspali*. *Claviceps paspali* represented 76% of the total fungal reads in the single forage sample. The complete DNA metabarcoding results dataset is available from the authors upon request.

Discussion

Definitive diagnosis of suspected cases of tremorgenic ergotism in grazing livestock is often challenging. In most cases, diagnoses are made using a preponderance of many types of data. History of access to poisonous plants, clinical signs consistent with ingestion of the suspected poisonous plant, body condition, histopathology, evidence of plant ingestion by detection of the bioactive compounds in gastrointestinal contents and/or animal tissues, and diet analysis by plant microhistological analyses and/or DNA metabarcoding may all be used to support a diagnosis of plant poisoning or an associated mycotoxicosis.

In cases of tremorgenic ergotism, clinical signs typically indicate neurologic disease, however, no characteristic lesions are produced and common veterinary laboratory methods (i.e., serum chemistry, histopathology, etc.) typically yield non-specific findings.[6] Furthermore, cases may occur sporadically, or in outbreaks affecting several animals at once such as in the present report. When investigating cases of suspected tremorgenic mycotoxicosis, the practitioner must 1) identify a history of exposure to pasture with evidence of *Claviceps* sp. ergot infection, 2) observe clinical signs such as exercise-exacerbated nervousness, belligerent attitude, tremors, ataxia and convulsions, and 3) rule out other differential diagnoses for these clinical signs.[14,15] In this report, ergotized (*Claviceps paspali*) and non-ergotized dallisgrass (*Paspalum dilatatum*) seedheads were collected from pasture 2 grazed by cows that developed clinical signs. The clinical signs and behavior observed in affected cows were consistent with those reported for dallisgrass staggers. DNA metabarcoding of the rumen contents and forage sample confirmed the ingestion of dallisgrass and that the dallisgrass was infected by *C. paspali*. The presence of identical, known tremorgenic indole diterpene compounds in both samples collected from pasture 2 where known exposure occurred, as well as from the rumen and abomasum of cows displaying clinical signs consistent with dallisgrass staggers provides strong evidence for *Claviceps paspali*-mediated ergotism as the cause of this outbreak. In this report, temporality of clinical signs relative to exposure also supports this diagnosis, as clinical signs of dallisgrass staggers typically occur within days after the animals have been exposed to the source. Spontaneous resolution of clinical signs following removal from the source is also supportive of this diagnosis.[14,15] Other differential diagnoses that were considered at some point in the investigation included salt toxicity, organophosphate toxicity and rabies virus. Spontaneous resolution of clinical signs as well as history and non-definitive brain sodium results ruled out salt toxicity. No history or evidence of organophosphate exposure could be identified, and both necropsy results and the unlikelihood of a large, simultaneous outbreak helped rule out rabies virus as a cause.

Tremorgenic indole diterpene mycotoxins are lipophilic molecules that, after ingestion, easily cross the blood-brain barrier, rapidly gaining access to the central nervous system.[16] Once within the central nervous system, tremorgenic mycotoxins are thought to act by interfering with inhibitory neuroreceptors and enhancing excitatory amino acid neurotransmitter release mechanisms.[14,17,18] Sheep, cattle and horses are particularly susceptible to the "grass staggers" syndrome that can occur within a few days of ingesting sclerotia of *Claviceps paspali* growing on the seedheads of mature dallisgrass (*Paspalum dilatatum*), bahiagrass (*paspalum notatum*), and bermudagrass (*Cynodon dactylon*). Ingestion of perennial ryegrass (*Lolium perenne*) infected with the endophyte *Neotyphodium lolii* has also been implicated in causing a similar "grass staggers" syndrome in sheep, cattle, horses and deer.[15,19] These conditions all exhibit self-limiting clinical signs that are usually reversible when the affected animal is removed from the tremorgen-contaminated source.[14] Conversely, annual ryegrass toxicosis, caused by highly toxic corynetoxins produced by the bacteria *Clavibacter toxicus*, produces similar clinical signs of incoordination, high-stepping gait and convulsions, but is often lethal due to the extensive cerebellar injury that results.[20] Tall fescue (*Lolium arundinaceum*) is perhaps the most common pasture forage associated with ergot-mediated mycotoxicosis events in livestock populations. Ergot alkaloids produced by the endophyte fungus *Epichloë coenophiala* result in vasoconstriction, leading to clinical signs such as loss of ear tips and tail switches, lameness and sloughing of hooves, fat necrosis, elevated body temperature, heat intolerance,

failure to shed winter hair coat, decreased feed intake and weight gain, reduced conception rates and milk production, and death.[21–26] Similar clinical signs may be seen in livestock that consume grains contaminated with ergot alkaloids produced by *Claviceps purpurea*.[27,28] Ergovaline is the most prevalent ergot alkaloid found in tall fescue, and is distinct from tremorgenic indole diterpene alkaloids. Thus, diagnostic approaches for ergot-mediated mycotoxicosis events involving tall fescue typically focus on the detection of ergovaline and other related ergot alkaloids.[26]

Epidemiologic investigations of suspected toxicosis events in livestock can be difficult. It is essential to collect a thorough history, perform physical examinations, develop a problem list, and consider appropriate differential diagnoses when investigating livestock toxicosis events.[29] A systematic approach to investigations of suspected toxicosis events helps the practitioner work towards a diagnosis, as well as rule-out other, non-toxic causes of morbidity or mortality. Timely description of morbidity/mortality or decreased production events is often one of the first steps in any outbreak investigation.[30,31] Epidemic curves of toxicosis events in livestock populations typically follow point-source or common-source patterns, and recording the occurrence of cases over time can provide evidence as to the nature of the outbreak.[31,32] Establishing a timeline of events prior to and during the discovery of cases may also point to changes in management, environment, diet, etc. that could be responsible for the outbreak. In many cases, the source of the toxin may not be found. For example, if a contaminated feedstuff is suspected as the source of the toxin, there may be no remaining unfed samples to evaluate. In these cases, diagnosis depends on collecting samples from affected animals. Because of the near infinite number of compounds that may result in toxicity when exposure to a sufficient dose occurs, a systematic investigation of the environment, diet and management should occur. The practitioner should also consider that although a particular toxin may be responsible for clinical signs, other factors contributing to the ingestion of the toxin may need to be addressed (e.g., if cattle are forced to consume poisonous plants due to a lack of appropriate forage). In the present report, the sudden onset of clinical signs in many animals within a short period of time strongly suggested toxin exposure. During the early stages of the investigation, the pasture movement history of the cattle was not clear; therefore, investigators searched pastures 3 and 4 believing they held the source of the toxin. It was not until all employees of the operation were questioned that the history of some cows having exposure to pasture 2 became clear, and the investigation was expanded to include this pasture.

This case provides an excellent example of the importance of interpreting ancillary testing critically and in context of both the clinical history of the case and the submitted sample. The frontal lobe submitted from J065 for sodium analysis yielded a concentration of 2,154 ppm. Sodium concentrations above 2,000 ppm in brain tissue have been reported to be diagnostic of salt toxicity in calves.[33,34] That being said, sodium concentration may vary considerably between anatomic regions of the brain by up to 35%.[25] As such, homogenization of an entire hemisphere is the ideal sample for brain sodium analysis; however, this may not be practical unless there is a very high clinical suspicion for salt toxicosis over other differentials for neurologic disease.[35] Standard practice during postmortem examination at many diagnostic laboratories is to collect the majority, if not all, of the brain for histopathologic examination to maximize chances of detecting and characterizing patchy or non-localizing CNS lesions. In the cases of J065 and F039, sections of cerebellum, brainstem including the obex, and frontal lobe were saved frozen for additional ancillary testing, with the remainder of the brain being fixed in formalin. Therefore, only the frontal lobe was submitted for sodium analysis with the cerebellum and brainstem reserved for additional testing as needed. Because of this, it is suspected that the elevated sodium is the result of uneven sodium distribution within the neuroparenchyma and other contributing factors such as dehydration, which is common in debilitated cattle and further elevates the sodium concentration.

When herd outbreaks occur, it is useful to collect and freeze feed samples or forage samples from the pastures where affected animals are located if a feed or forage toxicosis is suspected. Although analysis of gastrointestinal contents may be rewarding in detecting toxic compounds such as in this case, false negative results may occur if the compound is rapidly absorbed or metabolized following ingestion. Additionally, some compounds or agents may be labile at room temperature or even refrigeration temperature, so consultation with the diagnostic or toxicologic laboratory may guide appropriate sample handling based on the etiology of suspicion.

Outbreaks of dallisgrass staggers have also been reported in Spain and South Africa.[10,36,37] Similar to the observations in the present report, Ayala-Soldado et al. reported that when affected animals were handled in the pasture, clinical signs immediately worsened with animals displaying aggressiveness and often collapse if they were able to stand. Since cases of dallisgrass staggers are most often first observed and examined in the pasture, the practitioner should exercise caution with these animals as the stress associated with examination and physical restraint of the animal (i.e., ropes or halters) may result in death.

In the present report, serum chemistry values demonstrated non-specific indicators of muscle damage and inflammation. Ayala-Soldado et al. noted a general increase in the serum biochemistry values aspartate aminotransferase (AST) and creatine kinase (CK), as was seen in the individual serum chemistry values from animals in this report.[37] Both CK and AST are found in high concentrations in muscle tissue, and therefore are often used as general indicators of muscle damage in domestic animals.[38] Muscle damage associated with a combination of recumbency, ataxia, tremors and propensity to fall likely explain the increase in these enzymes. The nonspecific lesions at necropsy and findings on serum chemistry panels demonstrate the diagnostic difficulty of identifying dallisgrass staggers, though the findings ruled out other systemic infectious, inflammatory and neoplastic disease differentials. Perhaps the strongest evidence of dallisgrass staggers as the cause of the clinical signs observed in this case is the total resolution of clinical signs within days following the removal of the animals from contaminated pasture; as has been observed in other case reports as well.[36,37] The serum chemistry results for J065 displayed several other abnormalities (e.g., hypoalbuminemia, hypoglycemia, hypermagnesemia, etc.) that may be attributed to ocular fluid as the source of the chemistry values.[39–41] Postmortem autolysis may also be partially responsible for differences seen in the chemistry values of J065, as this cow had presumably been dead for several hours prior to discovery.

Interestingly, no reproductive consequences were observed in this herd in the days following resolution of clinical signs. Routine herd work (i.e., vaccinations, pregnancy examinations, etc.) was postponed for approximately 3 weeks to allow for complete recovery prior to the stress of gathering and working. When pregnancy examinations were performed on November 9, 2023, 21-days following discovery of the initial case, all cattle that had displayed clinical signs, regardless of whether they were treated or not, remained pregnant.

Conclusion

This report describes the epidemiologic investigation of an outbreak of ataxia, tremors and hyperexcitability in a cow-calf herd on pasture in Mississippi following ingestion of tremorgenic mycotoxins produced by *Claviceps paspali* in infected dallisgrass. Open communication between the farm staff, managers, clinicians, pathologists and toxicologists was essential to arriving at a definitive diagnosis in this case. Investigations of suspected toxicosis cases can be challenging, and consultation is recommended to narrow down differentials and guide appropriate testing. In this case, routine necropsy procedures ruled out many potential disease processes. Diagnostic necropsy procedures are typically affordable to the producer and offered through state diagnostic laboratory systems. While diagnostic laboratories may offer toxicology screening, the additional cost may be prohibitive to producers. The USDA-ARS Poisonous Plant Research Laboratory provides diagnostic services, particularly chemical analysis of suspected plant toxicosis cases in livestock, to producers and referring veterinarians on a case-by-case basis. Dallisgrass staggers can be difficult to diagnose due to the sporadic nature of its occurrence, the lack of specific necropsy and other diagnostic testing results, and the tendency of cases to improve without intervention if removed from the source. Prevention depends on pasture and grazing management, and there is no specific treatment for affected individuals beyond supportive care.

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Endnotes

[a] = BD Vacutainer® Lithium Heparin 10.0 mL tube, BD, Franklin Lakes, NJ

[b] = Michigan State University Veterinary Diagnostic Lab Toxicology Section

[c] = Keystone Scientific, Inc. Bellefonte, PA

[d] = Thermo Scientific, San Jose, CA

[e] = Jonah Ventures, Boulder, CO

References

1. Lemus R. Dallisgrass and Late Summer Ergot Toxicity. *Mississippi State University Extension Center for Forage Management and Environmental Stewardship - Forage News*. 2016;9(9). Accessed January 10, 2024. **https://extension.msstate.edu/sites/default/files/newsletter/forage-news/2016/forage_newsletter_sept2016.pdf**

2. Holt EC. Dallisgrass. *Texas Agricultural Experiment Station*. 1956;(829):1-16. Accessed April 21, 2024. **https://oaktrust.library.tamu.edu/handle/1969.1/86575**

3. Cole RJ, Dorner JW, Lansden JA, et al. Paspalum staggers: isolation and identification of tremorgenic metabolites from sclerotia of *Claviceps paspali*. *J Agric Food Chem*. 1977;25(5):1197-1201. **https://doi:10.1021/jf60213a061**

4. Brown HB. Life History and Poisonous Properties of *Claviceps paspali*. *J Agri Res*. 1916; VII(9):401-406.

5. Mayland HF, Cheeke PR. *Forage-Induced Animal Disorders*. Vol II.; 1995.

6. Nicholson SS. Southeastern Plants Toxic to Ruminants. *Vet Clin North Am Food Anim Pract*. 2011;27(2):447-458. **https://doi:10.1016/j.cvfa.2011.02.008**

7. Mostrom MS, Jacobsen BJ. Ruminant Mycotoxicosis. *Vet Clin North Am Food Anim Pract*. 2011;27(2):315-344. **https://doi:10.1016/j.cvfa.2011.02.007**

8. Osweiler G. *Mycotoxins in Forages and Their Impact on Animal Health*. 1994.

9. Uhlig S, Egge-Jacobsen W, Vrålstad T, Miles CO. Indole–diterpenoid profiles of *Claviceps paspali* and *Claviceps purpurea* from high-resolution Fourier transform Orbitrap mass spectrometry. *Rap Comm Mass Spect*. 2014;28(14):1621-1634. **https//doi:10.1002/rcm.6938**

10. Uhlig S, Botha CJ, Vrålstad T, Rolén E, Miles CO. Indole-Diterpenes and Ergot Alkaloids in *Cynodon dactylon* (Bermuda Grass) Infected with *Claviceps cynodontis* from an Outbreak of Tremors in Cattle. *J Agric Food Chem*. 2009;57(23):11112-11119. **https://doi:10.1021/jf902208w**

11. Dorner JW, Cole RJ, Cox RH, Cunfer BM. Paspalitrem C, a new metabolite from sclerotia of *Claviceps paspali*. *J Agric Food Chem*. 1984;32(5):1069-1071. **https://doi:10.1021/jf00125a033**

12. Galanti L, Shasha D, Gunsalus KC. Pheniqs 2.0: accurate, high-performance Bayesian decoding and confidence estimation for combinatorial barcode indexing. *BMC Bioinformatics*. 2021; Dec 22:1-6.

13. Edgar RC. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. BioRxiv. 2016 Oct 15:081257. **https://doi.org/10.1101/081257**

14. Evans TJ, Gupta RC. Tremorgenic mycotoxins. In: *Veterinary Toxicology: Basic and Clinical Principles*. Elsevier; 2012:1231-1238. **https://doi:10.1016/B978-0-12-385926-6.00107-1**

15. Burrows GE, Tyrl RJ. Poaceae Barnhart. In: *Toxic Plants of North America*. 2nd ed. John Wiley & Sons, Inc.; 2013:888-997.

16. Patterson DSP, Shreeve BJ, Roberts BA, Macdonald SM. *Verruculogen Produced by Soil Fungi in England and Wales*. Vol 42.; 1981. **https://journals.asm.org/journal/aem**

17. Norris PJ, Smith CCT, De Belleroche J, et al. Actions of Tremorgenic Fungal Toxins on Neurotransmitter Release. *J Neurochem*. 1980;34(1):33-42. **https://doi:10.1111/j.1471-4159.1980.tb04618.x**

18. Selala MI, Daelemans F, Schepens PJC. Fungal Tremorgens: The Mechanism of Action Of Single Nitrogen Containing Toxins - A Hypothesis. *Drug Chem Toxicol*. 1989;12(3-4):237-257. **https://doi:10.3109/01480548908999156**

19. Pfister JF, Cheeke PR. Natural toxicants in feeds, forages, and poisonous plants. *J Range Manage*. 1998;51:127. **https://api.semanticscholar.org/CorpusID:83134886**

20. Cheeke PR. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. *J Anim Sci*. 1995;73(3):909-918. **https://doi:10.2527/1995.733909x**

21. Hoveland CS, Schmidt SP, King Jr. CC, et al. Steer Performance and Association of *Acremonium coenophialum* Fungal Endophyte on Tall Fescue Pasture1. *Agron J*. 1983;75(5):821-824. **https://doi.org/10.2134/agronj1983.00021962007500050021x**

22. Jacobson DR, Miller WM, Seath DM, Yates SG, Tookey HL, Wolff IA. Nature of Fescue Toxicity and Progress toward Identification of the Toxic Entity. *J Dairy Sci*. 1963;46(5):416-422. **https://doi:10.3168/jds.S0022-0302(63)89066-9**

23. Ferguson TD, Vanzant ES, McLeod KR. Endophyte Infected Tall Fescue: Plant Symbiosis to Animal Toxicosis. *Front Vet Sci*. 2021;8. **https://doi:10.3389/fvets.2021.774287**

24. Poole DH, Lyons SE, Poole RK, Poore MH. Ergot alkaloids induce vasoconstriction of bovine uterine and ovarian blood vessels. *J Anim Sci*. 2018;96(11):4812-4822. **https://doi:10.1093/jas/sky328**

25. Leuschen B, Ensley S, Plummer P. Ergot toxicosis causing death in weaned beef calves. *Bov Pract*. 2014;48(2):134-138.

26. Evans TJ, Blodgett DJ, Rottinghaus GE. Fescue toxicosis. In: *Veterinary Toxicology: Basic and Clinical Principles*. Elsevier; 2012:1166-1177. **https://doi:10.1016/B978-0-12-385926-6.00115-0**

27. Canty MJ, Fogarty U, Sheridan MK, Ensley SM, Schrunk DE, More SJ. Ergot alkaloid intoxication in perennial ryegrass (*Lolium perenne*): an emerging animal health concern in Ireland? *Ir Vet J*. 2014;67(1):21. **https://doi:10.1186/2046-0481-67-21**

28. Thompson FN, Stuedemann JA, Hill NS. Anti-Quality Factors Associated with Alkaloids in Eastern Temperate Pasture. *J Range Manage*. 2001;54(4):474. **https://doi:10.2307/4003119**

29. Evans TJ. Diagnostic Challenges and Guidelines Pertaining to Suspected Ruminant Intoxications. *Vet Clin North Am Food Anim Pract*. 2020;36(3):509-524. **https://doi:10.1016/j.cvfa.2020.08.007**

30. Smith DR. Investigating Outbreaks of Disease or Impaired Productivity in Feedlot Cattle. *Vet Clin North Anim Food Anim Pract*. 2015;31(3):391-406. **https://doi:10.1016/j.cvfa.2015.05.003**

31. Smith DR. Field Disease Diagnostic Investigation of Neonatal Calf Diarrhea. *Vet Clin North Am Food Anim Pract*. 2012;28(3):465-481. **https://doi:10.1016/j.cvfa.2012.07.010**

32. Waldner CL, Campbell JR. Disease outbreak investigation in food animal practice. *Vet Clin North Am Food Anim Pract*. 2006;22(1):75-101. **https://doi:10.1016/j.cvfa.2005.12.001**

33. Dore V, Smith G. Cerebral Disorders of Calves. *Vet Clin North Am Food Anim Pract*. 2017;33(1):27-41. **https://doi:10.1016/j.cvfa.2016.09.004**

34. Osweiler GD, Carr TF, Sanderson TP, Carson TL, Kinker JA. *Water Deprivation-Sodium Ion Toxicosis in Cattle*. Vol 7.; 1995.

35. Romano MC, Higgs GE, Helm MN, Stefanovski D, Gaskill CL. Sodium distribution in the bovine brain. *J Vet Diag Invest*. 2021;33(2):384-387. **https://doi:10.1177/1040638720982989**

36. Botha CJ, Kellerman TS, Fourie N. A tremorgenic mycotoxicosis in cattle caused by *Paspalum distichum* (L.) infected by *Claviceps paspali*. *J S Afr Vet Assoc*. 1996;67(1):36-37.

37. Ayala-Soldado N, Lora-Benitez AJ, Mora-Medina R, Molina-Lopez AM, Artillo-Guimera JI, Moyano-Salvago MR. Tremorgenic mycotoxicosis in cattle, caused by *Claviceps paspali*. *Vet Med (Praha)*. 2022;67(12):638-643. **https://doi:10.17221/25/2022-VETMED**

38. Stampfli H, Oliver-Espinosa O. Clinical Chemistry Tests: Serum Enzymes. In: Smith BP, ed. *Large Animal Internal Medicine*. 5th ed. Elsevier Mosby; 2015:365-368.

39. Hanna PE, Bellamy JEC, Donald A. Postmortem Eye-fluid Analysis in Dogs, Cats and Cattle as an Estimate of Antemortem Serum Chemistry Profiles. *Can J Vet Res*. 1990;54(4):487-494.

40. Edwards G, Foster A, Livesey C. Use of ocular fluids to aid postmortem diagnosis in cattle and sheep. *In Pract*. 2009;31(1):22-25. **https://doi:10.1136/inpract.31.1.22**

41. McLaughlin PS, McLaughlin BG. Chemical analysis of bovine and porcine vitreous humors: correlation of normal values with serum chemical values and changes with time and temperature. *Am J Vet Res*. 1987;48(3):467-473.

