PEER REVIEWED

Multiple bovine abortions with evidence of fetal locoism from pre-clinically intoxicated dams

Michael J. Betley,[1] DVM, PhD; **Bryan L. Stegelmeier,**[2] DVM, PhD, DACVP; **Gene Niles,**[3] DVM, MS, DABVT; **Chad B. Frank,**[4] DVM, MS, DACVP
[1] Colorado State University, Fort Collins, CO 80523
[2] USDA Poisonous Plant Research Laboratory, Logan, UT 84322
[3] Colorado State Veterinary Diagnostic Laboratory, Rocky Ford Branch, Rocky Ford, CO 81067
[4] Colorado State University, Fort Collins, CO 80523
Corresponding author: Dr. Michael J. Betley; mikebetley@gmail.com

Abstract

Chronic consumption of specific plant species from the genera *Oxytropis*, *Astragalus*, *Ipomeoa*, and *Swainsonia*, commonly known as locoweeds, is well known to induce locoism in grazing animals. Locoism in cattle is characterized by sensory deficits, ataxia, behavior changes, loss of condition, and failure to grow. The toxic principle swainsonine is rapidly absorbed and distributed through the bloodstream to multiple organs where it inhibits cellular lysosomal alpha-mannosidase and Golgi mannosidase II, leading to lysosomal dysfunction and disruption of glycoprotein processing. Excessive mannose-rich oligosaccharide accumulations cause cytoplasmic vacuolation and cellular dysfunction. In addition to neurologic disease, chronic consumption of locoweeds is known to induce abortion and embryonic death in neurologically affected dams. We describe multiple cases of bovine abortion associated with chronic locoweed ingestion in non-clinical dams from a herd in southeastern Colorado. Neurons from the cerebral cortex, brainstem, cerebellum, and renal tubular epithelial cells displayed the characteristic cytoplasmic vacuolation observed in adult cases of locoism in the aborted animals. Although chronic ingestion of locoweed is widely known to cause early gestation embryonic death, late-term hydrops amnii, and fetal fluid accumulation, locoweed-induced abortion should be considered as a differential diagnosis for cases of non-infectious abortion in grazing cattle, even in the absence of maternal clinical signs. These findings indicate that the bovine fetus may be more sensitive to the abortive effects of swainsonine than previously thought.

Key words: bovine, fetus, locoweed, abortion

Résumé

L'ingestion chronique de certaines espèces de plantes appartenant aux genres *Oxytropis*, *Astragalus*, *Ipomeoa* et *Swainsonia*, communément appelées les oxytropis, est connue pour provoquer le locoïsme chez les animaux brouteurs. Le locoïsme chez les bovins se caractérise par des déficiences sensorielles, l'ataxie, des changements comportementaux, la perte de condition et le manque de croissance. La swainsonine, l'agent toxique, est rapidement absorbée et se rend via la circulation sanguine dans plusieurs organes où il inhibe l'alpha-mannosidase lysosomale cellulaire et la mannosidase II de Golgi causant la dysfonction lysosomale et un dérèglement du traitement des glycoprotéines. L'accumulation excessive d'oligosaccharides riches en mannose entraine la vacuolisation cytoplasmique et la dysfonction cellulaire. En plus de troubles nerveux, l'ingestion chronique d'oxytropis est connue pour induire l'avortement et la mortalité embryonnaire chez les femelles affectées neurologiquement. Nous décrivons plusieurs cas d'avortement chez les bovins associés à l'ingestion chronique d'oxytropis par des femelles asymptomatiques d'un troupeau dans le sud-est du Colorado. La vacuolisation cytoplasmique caractéristique que l'on observe chez les adultes atteints de locoïsme était présente dans les neurones du cortex cérébral, du tronc cérébral et du cervelet de même que dans les cellules épithéliales tubulaires rénales des animaux avortés. Même s'il est bien connu que l'ingestion chronique d'oxytropis cause la mortalité embryonnaire tôt en gestation, l'anasarque fœtale tard en gestation et l'accumulation de fluide fœtal, l'avortement causé par l'ingestion d'oxytropis devrait être considéré comme un diagnostic différentiel dans les cas d'avortement non-infectieux chez les bovins au pâturage même en l'absence de signes cliniques maternels. Ces observations indiquent que le fœtus bovin pourrait être plus sensible aux effets abortifs de la swainsonine qu'on ne l'avait pensé.

Introduction

Chronic ingestion of toxic locoweeds over a course of several weeks can induce locoism in grazing production animals and wildlife. As many locoweeds are native plants that

can dominate certain plant communities, locoweed-induced toxicity is common and has been considered the most significant poisonous plant problem for livestock producers in the western United States.[6] Grazing animals often prefer locoweed species during the fall, winter, and early spring when other palatable forage sources are not readily available. Clinical locoism is characterized by neurologic deficits including ataxia, sensory deficits, and behavioral changes as well as failure to thrive and loss of condition.[3] Ingestion of locoweed by pregnant dams is also associated with reproductive complications that include early embryonic death, abnormal developmental of placental cotyledons, delayed placentation, hydrops amnii, congenital fetal malformations, and abortion.[2] Together, the potential effects of locoweed consumption on production animals have extremely significant ramifications for producers.

Species from the genera *Astragalus* and *Oxytropis* are flowering perennial plants that occupy many plant communities in western rangelands.[14] Although there are greater than 350 plant species in the 2 genera, only 24 species have been documented to contain sufficient swainsonine, the toxic indolizidine alkaloid that induces locoism.[13] The swainsonine-containing species of *Astragalus* and *Oxytropis* in North America maintain a symbiotic relationship with fungal endophytes, and the endophytic fungus is solely responsible for producing the toxic swainsonine.[3] The toxin-producing endophyte is vertically passed from mother to daughter in the seed coat, and locoweed species with decreased levels of the endophyte produce minimal swainsonine.[18] Swainsonine is water soluble, and once ingested, is rapidly absorbed through the gastrointestinal tract into circulation.[15] Cells of multiple organs absorb swainsonine, and it inhibits the enzymes lysosomal alpha-mannosidase and Golgi alpha-mannosidase II, which are required for normal cellular function.[17] Inhibition of lysosomal alpha-mannosidase leads to the accumulation of mannose-rich oligosaccharides within the lysosome, and aggregation of unprocessed mannose in lysosomes produces cytoplasmic swelling and vacuolation observed histologically. Golgi alpha-mannosidase II is essential for N-glycan processing, and the inhibition of this enzyme by swainsonine results in improper glycoprotein processing. The severity of clinical disease depends on the dose-dependent degree of accumulation and amount of cellular dysfunction induced. Many different cell types in both adult cattle and fetuses are susceptible, but cytoplasmic vacuolation due to mannose accumulation is most often histologically prominent in the neurons of the central nervous system and epithelial cells of multiple organs, such as the kidney and endocrine organs.[5] Gross lesions are not typically observed in the central nervous system or other affected organ systems, but congestive heart failure can occur in adult cattle poisoned at high elevations.[9]

Case History

A herd of cross-bred beef cattle on open pasture in south-eastern Colorado suffered multiple late-term abortions in early spring of 2017. No evidence of clinical disease was observed in the dams that aborted. Two aborted fetuses were submitted to the Rocky Ford branch of the Colorado State Veterinary Diagnostic Laboratory for necropsy and diagnostic testing. Both fetuses had fully developed hair coats and partially inflated lungs, indicating that they were near full term, and histopathology of the lung confirmed inflation of the alveoli, consistent with attempts to breathe. Routine screening tests for infectious and non-infectious causes of abortion were performed on both fetuses. Testing for bovine viral diarrhea virus (direct fluorescent antibody test), infectious bovine rhinotracheitis (direct fluorescent antibody test), and *Neospora caninum* (PCR) was negative, and aerobic and anaerobic bacterial cultures failed to grow any pathogenic bacteria. No testing for *Leptospira* sp was performed. The concentration of nitrate within the aqueous humor was 10ppm (normal <20 ppm). Histological analysis of fetal brains identified marked cytoplasmic swelling and vacuolation in neurons of the brainstem and cerebral cortex (Figure 1, A-D). Similar lesions were discovered in many tubular epithelial cells in the cortices of the kidneys (Figure 1, E, F). These lesions were not specific for an etiology, but highly suggestive of a lysosomal storage disorder, and locoweed poisoning was considered as the top differential diagnosis. Prior to 2017, the herd had a documented history of locoism,

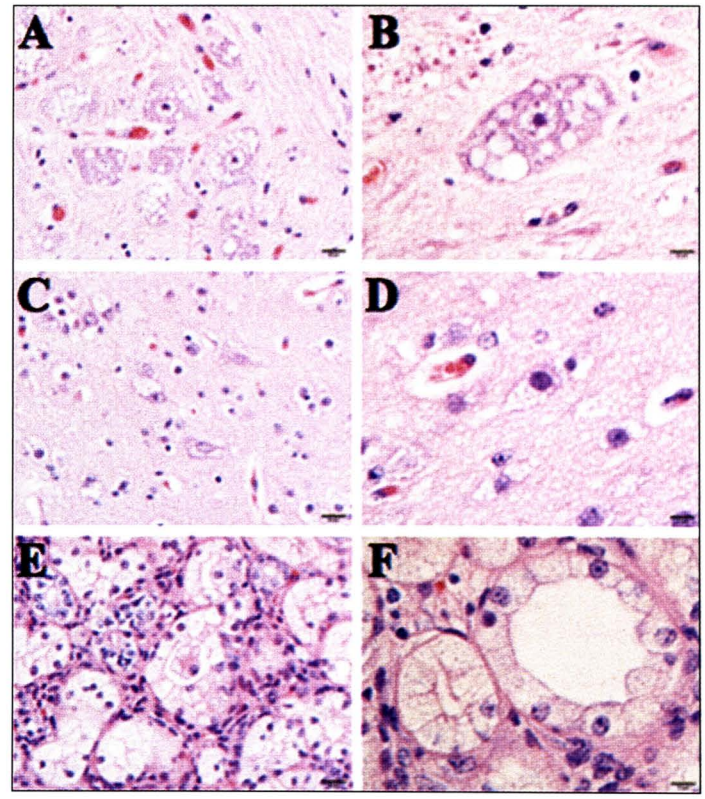


Figure 1. Cerebral cortex, brainstem, and renal pathology in aborted fetuses. Cytoplasmic vacuolation in brainstem neurons (A, B), cerebral cortical neurons (C, D), and renal tubular epithelium (E, F). A, C, E-500x, B, D, F-1000x.

and locoweed species had been tentatively identified on the property previously.

Suspicious plant samples from the affected ranch were collected and submitted to the USDA Poisonous Plant Research Laboratory for identification. The specimens were positively identified as Wooly locoweed (*Astragalus mollisimus*). Wooly locoweed is native to the Colorado plateau, and is well recognized as a swainsonine-producing species capable of inducing locoism in cattle.[14] Swainsonine concentrations within some toxic species can vary between geographically separated populations, and this is at least partially due to variations in the amount and strain of swainsonine-producing endophytes that infect locoweeds and other swainsonine containing plants.[6] Swainsonine was successfully isolated from the suspect plants using a commercially available solid-phase extraction column[a] and quantified using LC-MS/MS as described previously.[9] The swainsonine concentration was determined to be 0.3% of total mass, a concentration that has been shown to poison livestock.[14] As swainsonine crosses the placental barrier and locoweed-induced fetal lesions have been described, several attempts were made to identify and quantify swainsonine from fetal fluids, liver, and lung. No swainsonine was identified in tissues or fluids from either fetus; maternal serum was not available for analysis. Affected dams later developed neurological disease consistent with locoism in the weeks following the abortions.

Discussion

To our knowledge, the 2 cases presented here are the first to document late-term bovine abortion associated with minimal or subclinical maternal locoweed-induced neurologic disease. The affected cows did later develop clinical locoism.

The detection of swainsonine within serum or tissue from the dams and fetuses in this case would provide increased evidence of causation. The half-life of swainsonine in serum is relatively brief, approximately 20 hours,[17] and affected dams were constantly on pasture with locoweed species. However, locoweed may have been ingested intermittently. Due to the short serum half-life of swainsonine, previous consumption of locoweed, even a few days prior to abortion, could have resulted in fetal lesions without detectable serum swainsonine. Ingestion of 0.2 mg/kg of swainsonine/day by sheep for at least 14 days is sufficient to induce histologic lesions in multiple organs,[16] and with a concentration of 0.3% swainsonine, a 1100 lb (500 kg) cow would only need to consume 30 to 40 grams or several handfuls of locoweed each day to reach a dose of approximately 0.2 mg/kg/day. Additionally, the consumption preferences of animals on pasture could vary from day to day, and it is unknown what quantities of locoweed were consumed at different times. In multiple years prior to 2017, the herd had a documented history of clinical signs associated with locoism in the adult cattle, including abortions from dams with neurological deficits. Lastly, the fact that the dams in the herd developed clinical locoism in the weeks following the abortions lends support towards locoweed consumption as the presumptive cause of the abortions.

Swainsonine toxicosis represents a unique acquired lysosomal mannosidosis, but inherited mannosidoses have been documented in cattle as well. All cattle breeds are considered susceptible to locoweed-induced alpha mannosidosis, but autosomal recessive alpha-mannosidosis is documented only in Angus, Galloway, and Murray Grey cattle.[5] An autosomal recessive beta-mannosidosis is known to occur in Salers cattle.[5] In both of the inherited mannosidases, there is a mutation in the gene encoding either alpha or beta-mannosidase that results in functional mannosidase deficiency and lysosomal mannose accumulation. In contrast, toxic levels of swainsonine inhibit Golgi mannosidase II function. In both the inherited and induced conditions, the unifying feature is impaired glycoprotein processing that results in pathological lysosomal accumulation of mannose. The neuronal lesions in both the inherited and induced mannosidoses are histologically indistinguishable.

Clinical signs of locoism are most frequently observed in adult grazing cattle, whereas the manifestations of autosomal recessive mannosidoses are typically seen in calves only. Calves born with inherited alpha-mannosidosis demonstrate reduced growth and progressive ataxia, and most affected calves die by 18 months of age.[5] Calves born alive to dams poisoned with swainsonine may demonstrate small size, diminished growth, and behavioral abnormalities similar to the inherited mannosidoses. The herd in this case did not have genetics from cattle breeds affected by the inherited mannosidoses, and this observation decreased suspicion of inherited mannosidosis as a differential diagnosis. In the case of the inherited mannosidoses, a population history of weak calves with progressive neurologic disease would have been expected within the herd. Although adult cattle in the herd had developed clinical locoism in the past, no neurologic disease or other fetal abnormalities had been reported in calves. The observation of locoism in adult cattle, including the later development of clinical signs in the dams that aborted the affected fetuses, strongly supports locoweed poisoning as the cause of abortion and the histologic lesions in this case.

Locoweed species are not unique in their ability to induce abortion in cattle. Other abortifacient plants in the western United States include Ponderosa pine (*Pinus ponderosa*), broom snakeweed (*Gutierrezia sarothrae*),[8] and nitrate-containing plants grown in high nitrogen content soil,[5] but no other known plants associated with abortion induce the characteristic neuronal lesions of locoweed poisoning. Species of lupines (Genus *Lupinus*) grow throughout many of the western states and can cause fetal defects when consumed by pregnant dams.[10] Maternal lupine ingestion during days 40 to 80 of gestation results in fetal musculoskeletal defects including arthrogryposis, torticollis, and cranial abnormalities[2]. Histologic neuromuscular lesions are not observed with

lupine toxicosis, and lupine poisoning was not considered as a differential diagnosis in this case.

Conclusion

Although the clinical effects of locoweed consumption on the reproductive system and pregnancy are well documented, the abortions reported here indicate that the threshold required to induce abortion is potentially lower than previously thought. This report has implications for clinical veterinarians and diagnosticians that are regularly presented with cases of bovine abortion in regions where locoweed species are endemic. Although abortions caused by locoweed consumption are typically seen only in dams with clinical locoism, locoweed-induced abortion should be considered as a differential diagnosis for cases of non-infectious abortion in grazing cattle, even in the absence of maternal clinical signs.

Endnotes

[a]Strata-X-C polymeric strong cation exchange, Phenomenex, Torrance, CA

Acknowledgements

We would like to thank the USDA Poisonous Plant Research Laboratory for plant identification, and isolation and quantification of swainsonine content. We thank the CSU VDL histology laboratory for processing tissues and staining slides. The authors declare no conflicts of interest.

References

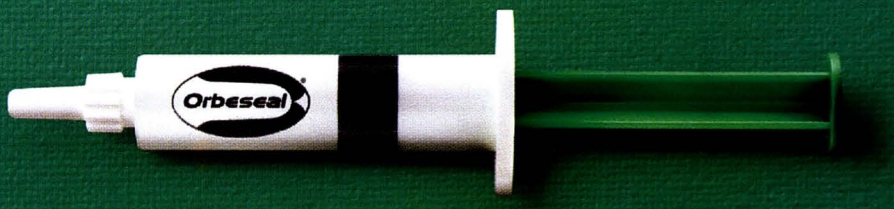
1. Abbott LC, Finnell RH, Chernoff GF, Parish SM, Gay CC. Crooked calf disease: A histological and histochemical examination of eight affected calves. *Vet Pathol* 1986;23:734-740.
2. Binns W, James LF. A congenital deformity in calves, similar to "crooked calf disease" has been experimentally produced by feeding heifers lupine and lead, in *Proceedings*. West Sect Am Soc Anim Prod 1961;12:1-3.
3. Braun K, Romero J, Liddell C, Creamer R. Production of swainsonine by fungal endophytes of locoweed. *Mycol Res* 2003;107:980-988.
4. Bunch TD, Panter KE, James LF. Ultrasound studies of the effects of certain poisonous plants on uterine function and fetal development in livestock. *J Anim Sci* 1992;70:1639-1643.
5. Cantile C, Youssef S. Storage diseases. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's pathology of domestic animals*. Vol 1. 6th ed. Philadelphia, PA: Elsevier Ltd, 2015;284-293.
6. Cook D, Gardner DR, Grum D, Pfister JA, Ralphs MH, Welch KD, Green BT. Swainsonine and endophyte relationships in *Astragalus mollissimus* and *Astragalus lentiginosus*. *J Agric Food Chem* 2011;59:1281-1287.
7. Gardner DR, Cook D. A comparison of alternative sample preparation procedures for the analysis of swainsonine using LC-MS/MS. *Phyt Anal* 2011;22:124-127.
8. Gardner DR, James LF, Panter KE, Pfister JA, Ralphs MH, Stegelmeier BL. Ponderosa pine and broom snakeweed: Poisonous plants that affect livestock. *J Nat Tox* 1999;8:27-34.
9. James LF, Hartley WJ, Nielsen D, Allen S, Panter KE. Locoweed (*Oxytropis sericea*) poisoning and congestive heart failure in cattle. *J Am Vet Med Assoc* 1986;189:1549-1556.
10. Lee ST, Panter KE, Gay CC, Pfister JA, Ralphs MH, Gardner DR, Stegelmeier BL, Motteram ES, Cook D, Welch, KD, Green BT, Davis TZ. Lupine-induced crooked calf disease: The last 20 years. *Rangelands*. 2008;6:13-18.
11. Molyneux RJ, James LF. Loco intoxication: Indolizidine alkaloids of spotted locoweed (*Astragalus lentiginosus*). *Science* 1982;216:190-191.
12. Panter KE, James LF, Stegelmeier BL, Ralphs MH, Pfister JA. Locoweeds: Effects on reproduction in livestock. *J Nat Toxins* 1999;8:53-62.
13. Ralphs MH, Creamer R, Baucom D, Gardner DR, Welsh SL, Graham JD, Hart C, Cook D, Stegelmeier BL. Relationship between the endophyte *Embellisia* spp and the toxic alkaloid swainsonine in major locoweed species (*Astragalus* and *Oxytropis*). *J Chem Ecol* 2008;34:32-38.
14. Ralphs MH, James LF. Locoweed grazing. *J Nat Toxins* 1999;8:47-51.
15. Stegelmeier BL, James LF, Panter KE, Molyneux RJ. Serum swainsonine concentration and alpha-mannosidase activity in cattle and sheep ingesting *Oxytropis sericea* and *Astragalus lentiginosus* (locoweeds). *Am J Vet Res* 1995;56:149-154.
16. Stegelmeier BL, James LF, Panter KE, Gardner DR, Pfister JA, Ralphs MH, Molyneux RJ. Dose response of sheep poisoned with locoweed (*Oxytropis sericea*). *J Vet Diag Invest* 1999;11:448-456.
17. Stegelmeier BL, James LF, Panter KE, Ralphs MH, Gardner DR, Molyneux RJ, Pfister JA. The pathogenesis and toxicokinetics of locoweed (*Astragalus* and *Oxytropis* spp) poisoning in livestock. *J Nat Toxins* 1999;8:35-45.
18. Valdez Barillas JR, Paschke MW, Ralphs MH, Child RD. White locoweed toxicity is facilitated by a fungal endophyte and nitrogen-fixing bacteria. *Ecology* 2007;88:1850-1856.

Experience did it.

NEW TRIES IT.

Experience matters. Especially when it comes to your cows. With ORBESEAL®, you get the only internal teat sealant proven by 15 years of results and university-backed studies. Which means you're working with the leader in teat sealant. Learn more at **orbeseal.com**.

Refer to the ORBESEAL label for complete instructions on proper administration at dry off and removal at freshening.



DAIRY WELLNESS MAKES A DIFFERENCE™

zoetis