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Clinical expression of perennial ryegrass (lolitrem-B) intoxication in New Zealand horses

A thesis presented in partial fulfillment of the requirements for the degree of Master of Veterinary Science

at Massey University, Manawatu, New Zealand.

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2010
Abstract

Perennial ryegrass staggers (PRGS) is a neurological mycotoxicosis caused by the ingestion of lolitrem-B. In this study, seven horses split into two separate groups were exposed to lolitrem-B by feeding them perennial ryegrass seed and hay containing 2 ppm lolitrem-B. Paired data was collected prior to and after two weeks exposure to lolitrem-B including video-documented neurological examination, clinical examination, brainstem auditory evoked (BAEP) and magnetic motor evoked (mMEP) potentials, blood and cerebrospinal fluid, and a frusemide challenge.

All horses developed tremor when exposed to lolitrem-B. The degree of tremor varied between individual horses and also depended on the level of activity, increasing during feeding and exercise. Using an ophthalmoscope a subtle, rapid (~5 Hz) tremor of the eyeball was detected in six of the seven horses. Subtle signs of ataxia were observed during handling, and motor dysfunction was exaggerated when blindfolded. Ataxia primarily involved a truncal sway and irregular, but predictable, limb placement that compensated for the lateralisation of the center of gravity. Results indicate that lolitrem-B may lengthen the peak V latency of BAEP traces. mMEPs also showed a lengthening in take-off latency and peak latency. The frusemide challenge revealed that renal K⁺ secretion was impaired significantly (p = 0.003) during the first 15 minutes after frusemide administration. During the treatment period resting heart rate increased significantly (p = 0.018) but stayed within normal values. No relevant changes were observed in respiration rate, rectal temperature, gastrointestinal auscultation or complete blood count, while changes in serum biochemistry require validation. No change was detected in urine lolitrem-B levels and although plasma lolitrem-B increased during the treatment period, levels did not correlate with the severity of clinical signs displayed.

This study provides a clearer appreciation of the clinical signs and variability of perennial ryegrass intoxication in horses. The clinical effects of lolitrem-B intoxication in horses primarily involve action-related tremors and symmetrical vestibular ataxia. Results from the frusemide challenge indicate that lolitrem-B disrupts renal large-conductance Ca²⁺-activated K⁺ channels, indicating a potential diagnostic avenue. Further research is required to establish the significance of increased mMEP and BAEP latencies.
Preface

The purpose of the study was to describe the clinical effects of lolitrem-B intoxication in horses in relation to those reported in ruminant species and to the function of BK channels. The effects of lolitrem-B were investigated in organ systems where BK channels are reported to play prominent roles. However, the scope was limited to include tests that are applicable to veterinary practice.

Acknowledgments

I would firstly like to thank my supervisor, Joe Mayhew for his enthusiasm, encouragement and guidance in this venture.

Louise and Andrew Scott, for the use of their facilities, their skills and for the many hours they spent working with the horses, I am extremely grateful. Also, thanks to Rob Mackay and Harry Carslake for their assistance with the trials.

I would also like to thank Lester Fletcher for his advice and support, and for arranging the harvest and transportation of the treatment feed.

Many thanks to those who were involved in the laboratory analysis of samples: Complete blood count, serum biochemistry and fractional excretions by Sue Pannifer (New Zealand Veterinary Pathology Ltd., IVABS, Palmerston North); in feed K⁺ analysis by Felicity Jackson (Animal Nutrition, IFNHH, Palmerston North); lolitrem-B determination in urine and plasma by Jan Sprosen (Toxinology group, Ag Research Ltd., Ruakura); determination of lolitrem-B levels in feed by Wade Mace (Grasslands Research Center, Ag Research Ltd., Palmerston North); aldosterone analysis by the Cardioendocrine Research Group, Christchurch.

Approval was obtained from the Massey University Animal Ethics Committee for the experiments described in this thesis (MUAEC Project 09/06).

The trial reported in this thesis was supported by funding from the Equine Trust, Equine partnership for Excellence.
To Aaron, my husband

Thank you for your understanding, encouragement and patience.
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**List of Abbreviations**

The following abbreviations are used within the main text and are defined when first used in each chapter.

- Ach: Acetylcholine
- AP(s): Action potential(s)
- BAEP(s): Brainstem auditory evoked potential(s)
- BK channel: Large-conductance Ca\(^{2+}\)-activated K\(^+\) channel
- BK\(_{\alpha/-}\): Mice lacking the gene that encodes the \(\alpha\)-subunit of the BK channel
- BK\(_{-\beta_{1/-}}\): Mice lacking the gene that encodes the \(\beta_{1}\)-subunit of the BK channel
- BM: Basilar membrane
- \([\text{Ca}^{2+}]_{i}\): Concentration of intracellular Ca\(^{2+}\)
- CAP(s): Compound action potential(s)
- CbTX: Charybdotoxin
- CCD: Cortical collecting duct
- CNS: Central nervous system
- CNT: Connecting tubule
- DRK channel: Delayed rectifying K\(^+\) channel
- EDTA: Ethylenediaminetetraacetic acid
- ELISA: Enzyme-linked immunosorbent assay
- ENa channel: Amiloride-sensitive epithelial Na\(^+\) channel
- EPSPs: Excitatory postsynaptic potentials
- (f)AHP: (Fast) afterhyperpolarisation
- FEK\(^+\): Fractional excretion of K\(^+\)
- FENa\(^+\): Fractional excretion of Na\(^+\)
- FMKS: Flow mediated K\(^+\) secretion
- GP: General proprioceptive
- IbTX: Iberiotoxin
- IHC(s): Inner hair cell(s)
- IK: Intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channel
- LMN(s): Lower motor neuron(s)
- mMEP(s): Magnetic motor evoked potential(s)
- \([\text{Na}^{+}]_{i}\): Intracellular concentration of sodium ions
- OHC(s): Outer hair cell(s)
- PC(s): Principal cell(s)
- PRG: Perennial ryegrass
- PRGS: Perennial ryegrass staggers
- RMP: Resting membrane potential
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<td>ROMK</td>
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<td>SK</td>
<td>Slow-conductance Ca²⁺-activated K⁺ channel</td>
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<td>TEA</td>
<td>Tetraethylammonium</td>
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<td>UMN(s)</td>
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Commentary on the DVD clips from neurological examinations.

The following is a commentary on the DVD movie clips, which are included in the DVD in the pocket of the back cover of this thesis. Readers are encouraged to refer to these as supplements to the descriptions provided in chapter 5.

**DVD 1: Muscle fasciculations**

Fasciculations are small involuntary muscle twitches that do not effect movement of a body part or segment and were observed as fine movement of the hair over the shoulder and pectoral muscles.

- **Horse #1**: After 9 days exposure to lolitrem-B, horse #1 showed a subtle fascicular tremor over the shoulder region while eating.
- **Horse #7**: Fasciculations predominantly involved the triceps and pectoral muscles after 5 days of lolitrem-B exposure. On day 12, fasciculations were prominent immediately after trotting and cantering on the lunge.

**DVD 2: Limb tremor and spasms**

- **Horse #2**: This horse demonstrated severe spasms of both forelimbs while eating, which increased in severity from a slight tremor on day 6 to severe spasms on day 9. If food were removed tremors would ease and when food was replaced the severity of tremor would again increase. Due to the amplitude of flexor spasm the heel bulbs would frequently leave the ground or the entire limb would be lifted during flexion and replaced again during the extension phase. Tremor of the right and left limb was reciprocal, in that as the right limb flexed the left limb would extend. However, an irregular sequence of right and left tremor was observed, rather than a 1:1 ratio. In this movie clip, forelimb tremor is most frequent in the left limb, which is bearing the least weight. Slowing the movie down allows an appreciation of the reciprocal motion and the order of joint flexion from distal to proximal, which gives an appearance of a ripple ascending the limb. Although tremor was most severe while eating, horse #2 also showed forelimb tremor during gait analysis particularly during pauses in movement or changes of direction.
- **Horse #1**: After 9 days of exposure to lolitrem-B, horse #1 displayed tremor in both pelvic and thoracic limbs. Pelvic limb tremor was subtle but occurred in both right and left limbs and was particularly noticeable at the fetlock when the heels were lifted as the horse pivoted or when the limb was lifted. As in the previous movie clip, this demonstrates that the tremor is not associated with
weight bearing. In the movie clip, the heels of the left forelimb remain grounded while the fetlock and carpus are flexed. During gait analysis, severe reciprocating left and right tremor of the thoracic limbs occurred, particularly when blindfolded.

- **Horse #3**: This movie clip was taken after 5 days exposure to lolitrem-B. In addition to slight tremor of the trunk, horse #3 showed tremor of the right forelimb that involved flexion of the carpus and fetlock, most obvious towards the end of the movie clip. The closer view displays tremor of the left forelimb.

- **Horse #5**: Recorded after 5 days of exposure, horse #5 showed tremor of the right forelimb and pectoral muscles immediately after lunging.

- **Horse #7**: On day 10 of the treatment period horse #7 showed a right forelimb tremor while at rest. Limb tremor was also observed when horse #7 was made to stand stationary after rapid movements.

**DVD 3: Vermiform tremor at the flank and over the ribs**

- Horse #5 demonstrated flank tremor, which appeared as irregular pulsations or punches from structures beneath the skin. Over the ribs the movement of the overlying skin was more undulating and wave-like, with ripples of muscle contractions.

**DVD 4: Initial signs of ataxia**

Initial signs of ataxia observed included a truncal sway at rest and a cautious gait at a walk with a wide-based placement of limbs and dishing of the limbs underneath the body during the protraction phase, particularly when blindfolded or with elevation of the head.

- **Horse #7**: The subtle, multidirectional sway of the trunk was best observed by watching the changing angle of the fetlock joints. Sway was noted at rest, when eating and before the onset of movement. Tremor of the right forelimb can also be seen on the movie clip from day 12.

- **Horse #6**: This horse scored 1/5 on day 14 of exposure and showed a very subtle craniocaudal sway, which was first observed on day 9.

- **Horse #3**: When blindfolded, horse #3 demonstrated a lateral truncal sway after turning. Ipsilateral limbs bore weight in synchrony not only changing angulations at the joints but also lifting as weight was transferred to the contralateral limbs. The right thoracic and pelvic limbs were observed to lift together. Horse #3 stabilised its stance by wide and forward placement of the left pelvic limb.
• **Horse #1:** Prior to lolitrem-B exposure, limbs were placed directly underneath the body. After 5 days of exposure to lolitrem-B, limb placement was lateral to the point of the shoulder and during the protraction phase the limb swung underneath the body in a curved path to be placed lateral once more. The wide-based gait slowed as the severity of PRGS progressed and resembled a waddle with thoracic and pelvic limbs moving in near synchrony. There was also a reduction in flexion of the joints during protraction i.e. hypometria.

• **Horse #5:** A wide based stance was adopted at rest and while eating. Blindfolding exaggerated the wide placement of limbs at a walk.

**DVD 5: Blindfolding**

• **Horse #2:** Prior to lolitrem-B exposure, horse #2 walked confidently when blindfolded and showed regular foot placement when walking in a straight line or turning. After 5 days exposure to lolitrem-B, blindfolding exposed an ataxic gait. When walking in a straight line with minimal prompts from the handler horse #2 was unable to maintain his line of direction. The trunk would sway, drift or lean to either side. The direction of the truncal sway was followed by irregular foot placements that would compensate for the lateral drift of the center of mass. Note the wide-based stance that was adopted at the end of movement. During circling, movement was regular when vision was not obscured. However, when blindfolded there was a delayed movement of the hindlimbs and the truncal leaned towards the inside of the circle. The outside hindlimb was brought under the body, compensating for the lean of the trunk; however, this resulted in the outside hindlimb interfering with the dorsal hoof of the inside hindlimb, which was subsequently rapidly moved to a wide position. Wide placement of the inside forelimb follows and the horse was stabilised by the wide-based stance. After 9 days of exposure, a mild ataxia is evident without blindfolding, with slight irregularities and jerkiness to movement and a tendency for the outside limb to circumduct during tight circling. However, ataxia was profoundly exaggerated by blindfolding. Horse #2 demonstrates a hesitancy and forelimb tremor at the onset of movement. While turning tightly on the left rein the trunk leaned inwards and the right fore and hindlimbs were suspended for a time. This was followed by circumduction of the outside hindlimb. Stumbling on the inside forelimb was followed by parallel placement of the outside forelimb and reciprocating tremor of both thoracic limbs, during which the inward and cranial lean of the trunk was exacerbated causing
imbalance. Hurried, lurching forelimb movement regained balance while the hindlimbs bounded forward in a wide-based, bunny-hopping gait.

- **Horse #1:** Prior to exposure, horse #1 had a confident and regular gait when blindfolded, whereas after 5 days exposure movement contained irregularities and involved increased movement of the trunk. During backing the trunk leaned to the right and when the left hindlimb was raised imbalance occurred but rapid movement of the limbs stabilised the horse. On day 9 exposure, horse #1 leant inward and cranial as it was led in a circle to the left resulting in imbalance and lurching of the body as rapid forelimb movement regained balance. During complex maneuvers, movement stammered and was awkward and jerky but limb placement was appropriate for the prompts given by the handler.

- **Horse #3:** This movie clip compares gait when the horse was and was not blindfolded on day 12 exposure.

- **Horse #7:** Pre-exposure, this horse did not maintain a straight line when walking blindfold; however, movement is not irregular and begins with turning of the neck, which the limbs follow without excessive sway of the trunk. When instructed to halt after turning, the horse did so without repositioning its limbs. Likewise on day 12 exposure when the horse was not blindfolded. However, when blindfolded and instructed to halt, the horse would sway and take stuttering steps to reposition limbs in a wide-based stance. He also showed interference of the hindlimbs and circumduction of the outside hindlimb during complex maneuvers.

- **Horse #5:** After 14 days of exposure to lolitrem-B there is a dramatic difference between gait when the horse was and was not blindfolded. This clip exemplifies how blindfolding exaggerated ataxia resulting in a slowed gait, increased sway of the trunk, irregular but predictable limb placement and a tendency to place limbs wide.

**DVD 6: Awkward stance**

After abrupt cessation of movement, horses would stand in awkward, abnormal positions and would only correct limb placement after a delay or when the next movement was initiated.

- **Horse #2:** On day 5 of lolitrem-B exposure horse #2 stood with forelimbs crossed and did not reposition limbs until forward movement was initiated.

- **Horse #4:** After turning in a circle the horse #4 stood with hindlimbs crossed.
• **Horse #2:** After lurching forward, limbs were placed wide with the right thoracic limb placed abnormally caudal. The forelimb was only repositioned square with the other forelimb after a delay.

**DVD 7: The serpentine maneuver**

The purpose of the serpentine maneuver was to test the ability of the horse to rapidly change the direction of movement of a limb while it is protracted. These movie clips demonstrate that when horses were given minimal direction from the handler irregular movement was observed. However, when the horse was lead in a serpentine manner, horses responded appropriately — changing the direction of limb placement according to the ordered movement of the head and neck.

- **Horse #2:** Day 5 exposure
- **Horse #5:** Day 14 exposure

**DVD 8: Lower motor neuron weakness was not displayed**

Horses did not demonstrate weakness during the tail pull, the tail and halter pull while circling, or thoracic limb hopping.

- **Horse #1:** During forelimb hopping, a weak horse has a tendency to tremble or collapse on the limb bearing weight. Horse #1 on day 9 demonstrates that an ability to bear weight well and resist pushing by the handler. Horses also exerted strong voluntary pull against the lateral tension applied during the tail pull. Horse #1, which was graded 3/5 on day 9 of lolitrem-B exposure demonstrates an ability to resist the tail pull.
- **Horse #7:** Pulling the tail while the patient is stationary initiates an extensor reflex in the hindlimb. This reflex is poor when there is a lower motor lesion at the level of L3-5. Horse #7 demonstrates a strong resistance to pull of the tail while standing still.

**DVD 9: Allodynia**

Horses showed increased response to a slap or threatening gesture at the withers.

- **Horse #1:** Day 9 exposure
- **Horse #3:** Day 12 exposure

Horses often startled or hesitated at gateways

- **Horse #2:** Day 9 exposure