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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

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New Zealand.

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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 Ca^{2+} -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.

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To Aaron, my husband
Thank you for your understanding,
encouragement and patience.

Table of Contents

Abstract.....	II
Preface and Acknowledgements.....	III
Dedication.....	IV
Table of Contents.....	V
List of Tables and figures.....	IX
Abbreviations.....	X
Commentary on the DVD clips from neurological examinations.....	XII
Chapter 1 Introduction.....	1
Chapter 2 Diagnosing perennial ryegrass staggers in horses.....	3
Chapter 3 The roles of large-conductance Ca ²⁺ -activated K ⁺ channels.....	13
Chapter 4 Research design.....	44
Chapter 5 Results.....	58
Chapter 6 Discussion.....	71
Chapter 7 Summary and Conclusion.....	107
References.....	111
Appendices.....	128

Chapter 1: Introduction	1
1.1 Problem Statement	1
1.2 Aim and Limitations	1
1.3 Thesis Structure	1
Chapter 2: Diagnosing perennial ryegrass staggers in horses	3
2.1 The etiology of perennial ryegrass staggers	3
2.2 The clinical presentation of perennial ryegrass staggers in ruminants and other herbivores	4
2.3 Literature on perennial ryegrass staggers in horses	6
2.4 The inadequacies of current methods of diagnosing perennial ryegrass staggers in horses	7
Chapter 3: The roles of large conductance Ca²⁺-activated K⁺ channels	13
3.1 The structure and functional diversity of large-conductance Ca ²⁺ -activated K ⁺ channels	13
3.2 Neural roles of large-conductance Ca ²⁺ -activated K ⁺ channels	14

3.2.1	Large-conductance Ca ²⁺ -activated K ⁺ channels may contribute to the negative resting membrane potential of excitable cells	17
3.2.2	The role of large-conductance Ca ²⁺ -activated K ⁺ channels in determining the rate and pattern of action potential firing in neurons	17
3.2.3	The disputed role of large-conductance Ca ²⁺ -activated K ⁺ channels in neurotransmitter release	20
3.2.4	The role of large-conductance Ca ²⁺ -activated K ⁺ channels in neural excitability depends on the entire biophysical context of the individual channel	23
3.3	Proposed mechanisms for tremor and ataxia displayed in lolitrem-B intoxication	24
3.3.1	Tremor	24
3.3.2	Cerebellar ataxia	25
3.3.3	Vestibular ataxia	27
3.3.4	General proprioceptive ataxia	29
3.3.5	Conclusion	30
3.4	Effects of lolitrem-B on auditory function	30
3.4.1	The role of large-conductance Ca ²⁺ -activated K ⁺ channels in inner hair cells and their influence on the temporal precision of auditory signals	31
3.4.2	The protective role of large-conductance Ca ²⁺ -activated K ⁺ channels in outer hair cells	34
3.4.3	Large-conductance Ca ²⁺ -activated K ⁺ channels do not influence endolymph properties	35
3.4.4	The proposed influence of lolitrem-B intoxication on auditory transmission	36
3.5	Explanations for the non-neurological clinical signs of perennial ryegrass staggers reported in ruminant trials, according to the roles of large-conductance Ca²⁺-activated K⁺ channels	36
3.5.1	The role of large-conductance Ca ²⁺ -activated K ⁺ channels in maintaining plateau potentials in lactotrophs and somatotrophs may explain the depressed serum prolactin levels and liveweight gain in hoggets grazing perennial ryegrass.	37
3.5.2	Ill-thrift, reduced liveweight gain and a change in faecal consistency may result from immune dysfunction	37
3.5.3	Lolitrem-B modifies gastrointestinal motility	38
3.6	The role of large-conductance Ca²⁺-activated K⁺ channels in renal K⁺ secretion	40
3.6.1	Localisation of large-conductance Ca ²⁺ -activated K ⁺ channels in the kidney	40
3.6.2	The role of large-conductance Ca ²⁺ -activated K ⁺	

channels associated with the β_1 -subunit in flow-mediated K^+ secretion	41
3.6.3 Potential compensation by homeostatic mechanisms	42
3.6.4 Lolitrem-B may interfere with flow-mediated K^+ secretion	42
Chapter 4 : Research Design	44
4.1 Hypothesis	44
4.2 Objectives	44
4.3 Brief overview of the method	45
4.4 Review of other methods	46
4.5 Research instrumentation	49
4.5.1 Clinical examination	49
4.5.2 Neurological examination	49
4.5.3 Complete blood count and serum biochemistry	50
4.5.4 Cerebrospinal fluid collection	50
4.5.5 Brainstem auditory evoked potentials	51
4.5.6 Magnetic motor evoked potentials	53
4.5.7 Frusemide challenge	54
4.5.8 Plasma and urine lolitrem-B levels	56
4.6 Tests that were excluded	56
4.7 Statistical analysis	57
Chapter 5: Results	58
5.1 Clinical description	58
5.2 Complete blood count, serum biochemistry and gross cerebrospinal fluid analysis	62
5.3 Electrophysiology	65
5.3.1 Brainstem auditory evoked potentials	65
5.3.2 Magnetic motor evoked potentials	67
5.4 Frusemide challenge	68
5.5 Lolitrem-B levels in body fluids – urine and plasma	70
Chapter 6: Discussion	71
6.1 How the clinical signs observed in this investigation compare to those reported in ruminant trials	71
6.2 Tremor and eye movements	73
6.2.1 Classifying muscle tremor	75
6.2.2 Eyeball tremor	77

6.3 Ataxia	79
6.3.1 The quality of ataxia did not resemble cerebellar ataxia	80
6.3.2 Blindfolding indicates that sensory input to the equilibrium centers is reduced by lolitrem-B intoxication	82
6.3.3 Further defining the nature of ataxia observed.	83
6.3.4 Involvement of the cerebellum in severe lolitrem-B intoxication	86
6.4 Horses intoxicated with lolitrem-B displayed allodynia and an increased resting heart rate	89
6.5 Complete blood count and serum biochemistry	91
6.6 Brainstem auditory evoked potentials	92
6.6.1 A delay in peak V latency indicates reduced temporal precision, but is unlikely to influence the behaviour of horses	93
6.6.2 Limitations of the research design that may have caused the lengthened latency of all peaks and produced traces that were unable to be interpreted	94
6.6.3 Brainstem auditory evoked potentials are unlikely to represent the vestibular dysfunction that resulted in ataxia in intoxicated horses	95
6.7 Magnetic motor evoked potentials	95
6.7.1 The neural pathways that mediate the magnetic motor evoked potentials in horses have not been determined and may involve extrapyramidal tracts	95
6.7.2 Blockade of large-conductance Ca ²⁺ -activated K ⁺ channels may reduce neural conduction in the extrapyramidal pathway	97
6.7.3 Limitations of the research design that may have caused the lengthened latency and produced traces that were unable to be interpreted	98
6.8 The extent of frusemide-induced K⁺ and Na⁺ excretion and basal levels of aldosterone were reduced after exposure to lolitrem-B	99
6.8.1 Mechanisms of reduced flow-mediated K ⁺ secretion by lolitrem-B	99
6.8.2 How lolitrem-B intoxication may influence frusemide-induced changes in fraction excretion of Na ⁺	100
6.8.3 Lolitrem-B intoxication might result in pathological electrolyte disturbances	102
6.8.4 Lolitrem-B induces a reduction of basal plasma aldosterone	102
6.9 Other effects of ergovaline	104

Chapter 7: Summary and Conclusion **106**

List of Tables and Figures

Table 2.1	Differentials for lolitrem-B intoxication in horses	10
Table 4.1	Scoring system used to evaluate the severity of lolitrem-B intoxication	46
Table 4.2	Previous clinical studies of perennial ryegrass staggers	48
Fig. 5.1	Wide-based stance	59
Table 5.1	Identification of horses used in the trial	60
Fig. 5.2	Abnormal awkward limb placement	61
Fig. 5.3	Heart rate and temperature during lolitrem-B exposure	62
Fig. 5.4	Serum biochemistry results	63
Fig. 5.5	Cerebrospinal fluid	64
Fig. 5.6	Repeatability of brainstem auditory evoked potentials	65
Fig. 5.7	Increased latency of peak V in brainstem auditory evoked potentials post-lolitrem-B exposure	66
Fig. 5.8	Increased latencies of magnetic motor evoked potentials post-lolitrem-B exposure	67
Fig. 5.9	Results from the frusemide challenge	69
Fig. 5.10	Plasma lolitrem-B values after lolitrem-B exposure	70
Fig. 6.1	Classification of muscle tremors by de Lahunta et al. (2006)	75
Table 6.1	Proposed scoring system for evaluating the severity of lolitrem-B intoxication in horses	88

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 _α ^{-/-}	Mice lacking the gene that encodes the α-subunit of the BK channel
BK-β ₁ ^{-/-}	Mice lacking the gene that encodes the β ₁ -subunit of the BK channel
BM	Basilar membrane
[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	Iberitoxin
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)
[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

ROMK	Renal outer medullary K ⁺ channels
SK	Slow-conductance Ca ²⁺ -activated K ⁺ channel
TEA	Tetraethylammonium
UMN(s)	Upper motor neuron(s)
VGC channel	Voltage-gated Ca ²⁺ channel
VGNa channel	Voltage-gated Na ⁺ channel
VHCII	Type II vestibular hair cells
WT	Wild-type
ΔFEK^+_{15}	The difference in the fractional excretion of K ⁺ between 0 and 15 min after frusemide administration
ΔFENa^+_{15}	The difference in the fractional excretion of Na ⁺ between 0 and 15 min after frusemide administration
ΔAld_{30}	The difference in plasma aldosterone between 0 and 30 min after frusemide administration
μSOLT	Familial microsaccadic eye oscillations and limb tremor

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 trunk 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