

## PREMISES-LEVEL RISK FACTORS FOR THE RECURRENCE OF EQUINE GRASS SICKNESS ON PREVIOUSLY AFFECTED ESTABLISHMENTS

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### Introduction

Equine grass sickness (EGS) or equine dysautonomia is a debilitating and rapidly fatal neurodegenerative disease of horses, the cause of which has still not been definitively identified. A clinically and pathologically identical disease, *mal seco*, is recognised in South America.

Clinical signs of EGS and *mal seco* are characterised as either acute, sub-acute or chronic according to the duration and severity of signs and are attributable to histologically evident disruption of the autonomic nervous system, particularly of the gastrointestinal tract. Clinical presentation is usually characterised by signs of mild colic, increased heart rate, muscle tremors, patchy sweating, difficulty in swallowing, reduced intestinal motility, weight loss and occasionally sudden death.

Epidemiological studies have consistently shown that the disease is associated with grazing, with peak diagnoses during the spring and early summer, although cases may occur in any month. Young animals and those that have recently moved premises and/or pasture are at significantly increased risk of disease. Studies have demonstrated a significantly increased risk of EGS associated with premises on which there have been previous cases and that this risk increased the more recently that cases had occurred. It has also been shown that there is a ten-fold reduction in risk for horses in contact with previous cases, consistent with the acquisition of immunity.

Although EGS recurs on previously affected premises, the factors that influence this have not been previously investigated, consequently practical methods of reducing the risk of such recurrence have not been available for horse owners. A questionnaire-based epidemiological study was, therefore, conducted to identify factors significantly associated with recurrence of EGS on premises. Knowledge of these factors may, through modifying certain management practices, help to reduce the frequency of cases on affected premises in the future.

### Materials and methods

Premises suffering cases of equine grass sickness (EGS) between January 1<sup>st</sup> 1997 and December 31<sup>st</sup> 2001 were identified from various sources and asked to complete a detailed questionnaire that included sections on disease history and horse, premises and pasture management. Recurrent premises were defined as those that had previous cases during a defined risk period. For each premise the total number of cases excluding the questionnaire case that occurred during the risk period, was calculated. Cases clustered within 30 days were treated as a single case. Data were analysed

using Poisson regression analysis and premises with risk periods less than 2 years were excluded. Standard forward stepwise multivariable analysis was conducted following preliminary univariable screening of variables.

## Results

Of 509 premises contacted during the study, 305 (60%) returned useable questionnaires and 100 of these (33%) were classified as recurrent premises. The median EGS incidence rate for 'recurrent' premises (0.25 cases/premises/year) was more than double that for 'non-recurrent' premises (0.10 cases/premises/year), a difference that was statistically significant ( $P < 0.0001$ ). However, examination of the distribution of numbers of horses on premises showed that 'recurrent' premises (median 15.5 horses/premises) had significantly ( $P < 0.0001$ ) greater numbers of horses than 'non-recurrent' premises (median 5 horses/premises). Median EGS incidence rates adjusted for horse numbers on premises actually showed no statistically significant difference ( $P = 0.52$ ) between 'recurrent' (2.06 cases/100 horses/premises/year) and 'non-recurrent' premises (2.10 cases/100 horses/premises/year).

Table 1 summarises the final multivariable Poisson regression model including several statistically significant interaction terms that were identified during model building. Controlling for other variables in the model, there was evidence for an increased rate of recurrence of EGS on premises associated with increasing horse numbers, the presence of younger animals, sand and loam soil types and use of mechanical droppings removal. There was evidence for a decreased rate associated with chalk and other soil types, removal of droppings by hand, grass cutting and ruminants grazing pasture.

## Conclusions

Among variables retained in the final multivariable Poisson regression model the majority demonstrated strong associations with recurrence of EGS on premises. As such they may provide novel insights into the pathogenesis of this disease. However, the identification of several significant interaction terms in the final multivariable model emphasises that considerable caution is required in interpreting the findings of this study and that these observations require specific hypotheses to be generated and subsequently tested in appropriately designed, controlled and randomised intervention studies.

Although access to grazing has historically been the major risk factor for EGS contention has since arisen as to the role that grass itself plays in the disease and the possibility that there is some specific aetiological factor within the soil. Many of the findings of this study are complementary to the theory that EGS is a toxico-infectious form of botulism. Several of the significant factors identified may relate to soil disturbance and consequent soil contamination of grass, thereby increasing the rate of exposure of grazing horses to the *Clostridium botulinum* bacterium that resides in soil. Results agree with earlier studies that the presence of younger, probably non-immune horses on premises increases the rate of recurrence of EGS.

Variable	Category	Coefficient	Standard error	Rate Ratio	95% CI		P-value
					lower	upper	
<i>Intercept</i>		.57	.28				
Number of horses on the premises	1-5			reference			<b>*&lt;0.0001</b>
	6-10	.08	.37	1.08	0.52	2.22	0.838
	11-15	.06	.32	2.90	1.55	5.42	0.001
	16-20	.46	.35	11.7	5.91	23.1	<0.001
	21-40	.27	.30	3.56	1.98	6.40	<0.001
	41+	.97	.31	7.18	3.92	13.2	<0.001
Presence of horses <2 y.o.	No			reference			<b>*0.0001</b>
	Yes [2YO]	.53	.24	1.70	1.06	2.71	0.027
Soil type	Clay			reference			<b>*&lt;0.0001</b>
	Sand	.36	.20	1.43	0.98	2.10	0.067
	Chalk	.48	.61	0.23	0.07	0.76	0.016
	Loam	.74	.21	2.11	1.40	3.16	<0.001
	Other	.98	.44	0.38	0.16	0.90	0.027
Method of droppings removal [REM]	Not removed			reference			<b>*&lt;0.0001</b>
	By hand [1]	.71	.49	0.18	0.07	0.48	0.001
	Mechanically [2]	.02	.47	2.76	1.10	6.94	0.031
Pasture cut	Not cut			reference			<b>*&lt;0.0001</b>
	Cut [CUT]	.15	.32	0.12	0.06	0.22	<0.001
Other domestic animals on the pasture [DOM]	None			reference			<b>*&lt;0.0001</b>
	Ruminants [1]	.22	.41	0.11	0.05	0.24	0.001
	Birds/fowl [2]	.09	.30	0.91	0.50	1.65	0.760
	Other [3]	.37	.03	0.09	0.01	0.70	0.021
[REM]*[2YO]	[REM 1]*[2YO]	.22	.50	3.40	1.27	9.11	0.015
	[REM 2]*[2YO]	.54	.42	0.58	0.26	1.32	0.196
[REM]*[CUT]	[REM 1]*[CUT]	.22	.46	3.39	1.37	8.38	0.008
	[REM 2]*[CUT]	.26	.40	1.30	0.59	2.84	0.515
							<b>*0.023</b>

[DOM]*[CUT]	[DOM	03	.48	7.65	2.99	19.6	<0.001
	1]*[CUT]						
	[DOM	73	.48	2.07	0.81	5.28	0.128
	2]*[CUT]						
	[DOM	91	.06	18.3	2.29	147	0.006
	3]*[CUT]						
							<i>*&lt;0.000</i>
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\*Likelihood ratio statistic (LRS)  $\chi^2$  P-value

**Table 1: Final multivariable Poisson regression model including interaction terms for recurrence of EGS on previously affected premises, showing coefficient estimates and their standard errors, rate ratios and their 95% confidence intervals and corresponding Wald and LRS  $\chi^2$  P-values**

As a result of these findings, it is tempting to suggest possible protective control measures for premises affected with EGS, such as good pasture management, co-grazing of ruminants and the avoidance of pasture sweepers and domestic birds. However, these interventions cannot be entirely justified from this single, retrospective study in which there was evidence of significant effect modification between various factors. The results require corroboration by further, independent studies and in future, specific hypotheses need to be generated and then tested rigorously in carefully designed and conducted intervention studies.