

General Articles

An epidemiological study of risk factors associated with the recurrence of equine grass sickness (dysautonomia) on previously affected premises

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Summary

Reasons for performing study: The reasons why equine grass sickness (EGS) recurs on premises are unknown and, consequently, practical methods for reducing the risk of recurrence are not available.

Objectives: To identify risk factors associated with recurrence of EGS on premises and to gain possible insights into the pathogenesis of the disease.

Methods: Data on disease history and risk factors were collected by postal questionnaire from premises with EGS cases between 1st January 1997 and 31st December 2001. Data on variation in rates of recurrence of EGS for different risk factors were analysed using Poisson regression analysis.

Results: Of 509 premises contacted, 305 (60%) returned useable questionnaires and 100 of these (33%) were classified as 'recurrent' premises. An overall median incidence rate for EGS of 2.1 EGS incidents/100 horses/premises/year was recorded. There was an increased rate of recurrence with higher numbers of horses, presence of younger animals, stud farms and livery/riding establishments, loam and sand soils, rearing of domestic birds and mechanical droppings removal. The rate of recurrence decreased with chalk soil, cograzing ruminants, grass cutting on pastures and removal of droppings by hand. Several statistically significant interactions were identified.

Conclusions: Many of the findings are consistent with the theory that EGS is a toxico-infectious form of botulism. Several of the significant factors identified may directly or indirectly relate to soil disturbance and consequent soil contamination of grass, thereby increasing the rate of exposure of grazing horses to *Clostridium botulinum*, which resides in soil.

Potential relevance: Identification of potentially modifiable risk factors may, ideally following validation in appropriately designed, controlled and randomised

intervention studies, lead to practical measures to reduce the incidence of EGS on previously affected premises.

Introduction

Grass sickness (EGS; equine dysautonomia) is a debilitating and frequently fatal neurodegenerative disease of horses, the cause of which has still not been definitively identified. The use of the term 'grass sickness' to describe the disease undoubtedly arose from the fact that it almost exclusively affected grazing horses (Begg 1936; Gilmour and Jolly 1974).

Grass sickness was first recognised in Scotland in the early 1900s (Tocher *et al.* 1923; Tocher 1924; Begg 1936; Greig 1942; McCarthy *et al.* 2001) but there are also reports of the disease from areas of England and Wales around the same time (Greig 1942; McCarthy *et al.* 2001). Since then, cases have been diagnosed in several mainland European countries (McCarthy *et al.* 2001) and a clinically and pathologically identical condition, *mal seco*, is recognised in South America (Uzal and Robles 1993; Araya *et al.* 2002).

Clinical signs of grass sickness are characterised as either acute, subacute or chronic according to the duration and severity of signs (Doxey *et al.* 1991a) and are attributable to histologically evident disruption of the autonomic nervous system, particularly of the gastrointestinal tract (Obel 1955; Mahaffey 1959; Barlow 1969; Scholes *et al.* 1993; Doxey *et al.* 2000; John *et al.* 2001). Clinical presentation of EGS is typically 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 (Tocher *et al.* 1923; Tocher 1924; Greig 1942; Doxey *et al.* 1991a; Milne 1996). Horses with the mildest, chronic form may be nursed successfully to recovery (Doxey *et al.* 1995), but all acute and subacute cases are incurable. Repeated episodes of EGS in the same animal are extremely rare and histologically confirmed disease has not been reported more than once in affected horses (B.C. McGorum, unpublished data). For the

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purposes of this study, recurrence of disease refers solely to repeated episodes of EGS on the same premises.

Several different theories have been proposed for the cause of EGS, including insects, fungi, filterable viruses, toxic plants and bacterial toxins (Tocher *et al.* 1923; Tocher 1924; Begg 1936; Greig 1942; McCarthy *et al.* 2001). The currently favoured theory, with both historical and modern evidence to support it, is that EGS is a form of toxico-infectious botulism, resulting from *Clostridium botulinum* producing toxin locally within the horse's intestinal tract.

Tocher first proposed botulism as the underlying cause of EGS and this theory was supported by evidence of statistically significant ($P < 0.000001$) protection against death by vaccination with an antitoxin neutralised botulinum toxin vaccine (Tocher *et al.* 1923; Tocher 1924; Wood *et al.* 1999). However, Tocher's theory lost favour until recently, when a significant association between presence of both the organism and *C. botulinum type C* toxin in gastrointestinal contents was shown in histologically confirmed EGS cases compared with apparently healthy controls (Hunter *et al.* 1999). Further studies have shown that EGS cases have significantly lower serum antibody titres to *C. botulinum* and its *type C* toxin than horses that either have been in contact with EGS affected horses or have grazed frequently affected pasture (Hunter and Poxton 2001; McCarthy 2002; McCarthy *et al.* 2004).

Several epidemiological studies of EGS have been conducted (Gilmour and Jolly 1974; Doxey *et al.* 1991b; Wood *et al.* 1998; McCarthy 2002; McCarthy *et al.* 2004). Studies consistently show that EGS is associated with grazing. Although cases may occur in any month, peaks in diagnoses occur during the spring and early summer (Doxey *et al.* 1991b; Wood *et al.* 1998). Young horses and those that have recently moved premises and/or grazing are at significantly increased risk of EGS (Gilmour and Jolly 1974; Wood *et al.* 1998; McCarthy 2002; McCarthy *et al.* 2004). Gilmour and Jolly (1974) and Wood *et al.* (1998) both identified a significantly increased risk of disease associated with previously affected premises and this risk increased the more recently that cases had occurred (Table 1). Wood *et al.* (1998) also showed a 10-fold reduction in risk for animals in contact with previous cases.

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

TABLE 1: Numbers of cases and controls and corresponding odds ratios for categories of 'time since the last case on the premises' in 2 epidemiological studies of equine grass sickness (EGS)

	No previous cases	Time since last EGS case		
		>5 years*	2-5 years	<2 years
<i>Gilmour and Jolly (1974)</i>				
EGS cases	44	12	8	26
Controls	1851	242	175	286
Odds ratio	1.0 (Ref.)	2.1	1.9	3.8
<i>Wood et al. (1998)</i>				
EGS cases	78	24	9	24
Controls	166	31	13	16
Odds ratio†	1.0 (Ref.)	1.3	2.5	6.7

*Or not known. †Odds ratios after accounting for matching, taken from Wood *et al.* (1998). Ref. = Referent.

Materials and methods

Premises recruitment

Premises that had suffered at least one EGS case (diagnosed in grazing animals on the basis of typical clinical signs and/or histopathology of ileal biopsy and/or *post mortem* samples) since the beginning of 1997 were identified from a variety of sources including the databases of the Animal Health Trust, Equine Grass Sickness Fund, Edinburgh Veterinary School and other UK veterinary faculties, laboratories and practices. Data collection started in October 1999, with premises that had suffered cases since 1st January 1997 being recruited retrospectively, and premises that had EGS cases after October 1999 being prospectively recruited up to the end of December 2001 when data collection ended.

Data collection

A detailed questionnaire (available on request from the corresponding author or at www.aht.org.uk) that was 11 pages long and divided into 5 sections was used to collect data from affected premises. *Section 1* asked for name, address and contact details and *Section 2* collected details on the EGS disease history, including numbers and dates of previous cases. *Section 3* asked about horse and premises details, including the numbers and ages of horses and type and size of premises, available housing, grazing and water sources. *Section 4* collected horse management details including how horses were kept, fed and treated with anthelmintic drugs and *Section 5* asked about pasture management practices including details of faeces removal, harrowing, fertilising, grass cutting, reseeding and other animals that were kept. Information from the original questionnaires was entered manually into a Microsoft Access database, with different levels of variables being variously coded for future analyses.

Definitions of 'recurrent premises' and 'risk periods'

The study was designed specifically to investigate risk factors associated with recurrence of grass sickness on premises over prolonged periods. Multiple cases occurring on the same premises within a short period of each other could not therefore reasonably be treated as true recurrences but rather as clusters probably subjected to the same predisposing risk factors as one another. For the purposes of appropriate analysis, such clusters could not be considered as independent and, consequently, cases that occurred within 30 days of each other on the same

TABLE 2: Summary of median incidence rates and horse numbers for all, recurrent and nonrecurrent premises

	Premises			P value*
	All	Recurrent	Nonrecurrent	
No. premises	305	100	205	
Median incidence (incidents/premises/year)	0.10	0.25	0.09	<0.0001
Median no. horses	8	15.5	5	<0.0001
Median adjusted incidence (incidents/100 horses/premises/year)	2.10	2.06	2.11	0.52

*Wilcoxon rank-sum test P value for differences between recurrent and nonrecurrent premises.

TABLE 3: Numbers and proportions of recurrent and nonrecurrent premises for different categories of premises and horse management variables and rate ratios, 95% confidence intervals and corresponding Wald χ^2 P values from univariable Poisson regression analyses

Variable	Category	Recurrent premises; n (%)	Nonrecurrent premises; n (%)	Rate ratio	95% CI		P value
					Lower	Upper	
No. horses on the premises	1–5	21 (17)	104 (83)	Referent			
	6–10	12 (21)	45 (79)	1.80	0.97	3.32	0.061
	11–15	17 (47)	19 (53)	7.09	4.25	11.83	<0.001
	16–20	12 (57)	9 (43)	9.03	5.26	15.49	<0.001
	21–40	20 (54)	18 (46)	8.55	5.24	13.97	<0.001
	41+	18 (64)	10 (36)	15.54	9.73	24.81	<0.001
Presence of horses age <2 years	No	41 (22)	142 (78)	Referent			
	Yes	59 (49)	61 (51)	3.41	2.63	4.43	<0.001
Premises type	Farm	27 (29)	67 (71)	Referent			
	Livery/Riding	30 (45)	37 (55)	2.87	2.06	4.01	<0.001
	Stud	20 (69)	9 (31)	2.70	1.82	4.00	<0.001
	Rented	8 (20)	32 (80)	0.68	0.37	1.25	0.220
	Other	15 (20)	60 (80)	1.22	0.83	1.79	0.313
No. stables	None	17 (28)	44 (72)	Referent			
	1–5	18 (18)	80 (82)	0.55	0.34	0.91	0.019
	6–10	20 (32)	42 (68)	1.34	0.86	2.10	0.195
	11–20	26 (50)	26 (50)	2.45	1.62	3.70	<0.001
	21+	19 (59)	13 (41)	4.47	3.00	6.69	<0.001
Water source while housed	Mains	70 (33)	144 (67)	Referent			
	Well	12 (60)	8 (40)	1.40	0.94	2.08	0.095
	Other	12 (27)	32 (73)	0.79	0.55	1.14	0.209
Water source while grazing	Mains	43 (28)	113 (72)	Referent			
	Stream	12 (38)	20 (62)	0.98	0.62	1.53	0.917
	Well	7 (41)	10 (59)	2.32	1.55	3.48	<0.001
	Mains + stream	24 (46)	28 (54)	1.69	1.24	2.31	0.001
	Other	14 (30)	32 (70)	1.22	0.85	1.75	0.276
Grazing type	Permanent	65 (28)	169 (72)	Referent			
	Ley	23 (52)	21 (48)	1.78	1.32	2.41	<0.001
	Hill/moor	11 (42)	15 (58)	1.74	1.21	2.51	0.003
Soil type	Clay	30 (27)	83 (78)	Referent			
	Sand	22 (37)	37 (63)	1.14	0.82	1.57	0.433
	Chalk	5 (29)	12 (71)	0.23	0.07	0.73	0.013
	Loam	24 (46)	28 (54)	1.36	0.99	1.88	0.059
	Other	9 (28)	23 (72)	0.49	0.28	0.88	0.017
Supplementary hay fed in spring	No	29 (34)	59 (67)	Referent			
	Yes	71 (35)	132 (65)	2.14	1.54	2.97	<0.001
Supplementary hay fed in summer	No	63 (34)	125 (66)	Referent			
	Yes	37 (36)	65 (64)	1.71	1.34	2.19	<0.001
Supplementary spring concentrate	No	25 (34)	49 (66)	Referent			
	Yes	75 (35)	142 (65)	1.24	0.92	1.67	0.152
Supplementary summer concentrate	No	43 (34)	82 (66)	Referent			
	Yes	57 (34)	109 (66)	1.67	1.28	2.17	<0.001
Anthelmintic frequency	≥6 monthly	11 (27)	30 (73)	Referent			
	3 monthly	43 (32)	92 (68)	2.68	1.54	4.67	<0.001
	2 monthly	35 (35)	66 (65)	3.08	1.76	5.38	<0.001
	<2 monthly	10 (43)	13 (57)	2.88	1.48	5.59	0.002

premises were treated as a single EGS incident. It is important that this assumption is considered in interpretation of the results presented.

‘Recurrent premises’ were defined as having at least one previous EGS incident (i.e. excluding the incident for which the questionnaire was sent, referred to as the ‘questionnaire incident’) during a defined risk period. For each premises, a risk period was calculated that was taken from the date of completion of the questionnaire back to either an arbitrary start date of 1st January 1990 or the date that the owner took up residence on the premises if this was after the beginning of 1990.

Calculation of ‘incidence rates’

Overall EGS incidence rates, expressed in ‘numbers of EGS incidents per year’, were initially calculated for each premises from the disease history, by dividing the total number of incidents (including the ‘questionnaire incident’ but with cases that occurred within 30 days of each other being treated as a single incident) by the risk period expressed in years. Adjusted incidence rates, expressed in numbers of EGS incidents/100 horses/year, were also calculated, with adjustment made for the number of horses on premises. This was based on the assumption that horse numbers on

TABLE 4: Numbers and proportions of 'recurrent' and 'non-recurrent' premises for different categories of pasture management variables and rate ratios, 95% confidence intervals and corresponding Wald χ^2 P values from univariable Poisson regression analyses

Variable	Category	Recurrent premises; n (%)	Nonrecurrent premises; n (%)	Rate ratio	95% CI		P value
					Lower	Upper	
Faeces removed?	Not removed	56 (31)	122 (69)	Referent			
	Removed	43 (34)	82 (66)	1.54	1.21	1.96	0.001
Method of faeces removal	Not removed	56 (31)	122 (69)	Referent			
	By hand	20 (21)	76 (79)	0.53	0.36	0.79	0.001
	Mechanically	23 (79)	6 (21)	4.30	3.30	5.60	<0.001
Pasture harrowed?	Not harrowed	49 (30)	117 (70)	Referent			
	Harrowed	50 (37)	85 (63)	0.97	0.76	1.24	0.819
Pasture fertilised?	Not fertilised	23 (20)	90 (80)	Referent			
	Fertilised	74 (40)	113 (60)	2.06	1.53	2.77	<0.001
Fertiliser type	None	23 (20)	90 (80)	Referent			
	Nitrogen-based	35 (32)	74 (68)	1.85	1.37	2.52	<0.001
	Other	39 (50)	39 (50)	1.67	1.20	2.33	0.002
Pasture cut?	Not cut	27 (28)	68 (72)	Referent			
	Cut	72 (35)	135 (65)	0.76	0.59	0.98	0.033
Pasture reseeded?	Not reseeded	50 (27)	132 (73)	Referent			
	Reseeded	47 (40)	70 (60)	1.16	0.91	1.48	0.235
Reseeded how long ago?	Not reseeded	50 (27)	132 (73)	Referent			
	<5 years ago	35 (48)	38 (52)	1.49	1.15	1.93	0.002
	>5 years ago	9 (23)	30 (77)	0.43	0.25	0.74	0.002
Other domestic animals on the pasture	None	29 (28)	75 (72)	Referent			
	Ruminants	46 (32)	97 (68)	0.70	0.53	0.93	0.014
	Birds/fowl	11 (48)	12 (52)	1.61	1.08	2.41	0.020
	Other	11 (36)	20 (64)	1.43	0.99	2.08	0.056

premises were largely static over time. Statistically significant differences between 'recurrent' and 'nonrecurrent' premises in the incidence rate, number of horses and adjusted incidence rate were investigated using the nonparametric Wilcoxon rank-sum test.

Poisson regression analyses

For the main analyses, the 'questionnaire incident' was excluded from the total number of EGS incidents so that the total would be equivalent to the number of previous incidents (i.e. recurrences). The structure of these data was events (recurrent EGS incidents) over time (risk period); therefore, an appropriate statistical approach was Poisson regression analysis (Frome 1983). However, to avoid biased rate measures due to overly short follow-up periods, 7 premises with risk periods less than 2 years were excluded from these analyses.

Univariable analyses: Each variable (Tables 3 and 4) was tested individually for the significance of its association with the recurrence of disease during the risk period using univariable Poisson regression (Stata software)¹.

Multivariable analyses: To control for confounding and effect modification between variables, those that were associated significantly with recurrence of disease at $P < 0.275$ in univariable analyses were used in multivariable Poisson regression analysis (Hosmer and Lemeshow 1989). The reason for choosing a P value > 0.05 was in order to avoid unnecessary early exclusion of significant risk factors whose effects may be masked by confounding variables in univariable analyses. Multivariable modelling was conducted using a forward stepwise approach (Hosmer and Lemeshow 1989). Variables were retained if they

were associated with disease (Wald χ^2 : $P \leq 0.05$) or their inclusion resulted in a significant improvement in the overall fit of the model as measured by the likelihood ratio statistic (LRS χ^2 : $P \leq 0.05$). The final model was examined for biologically meaningful 2-way interaction terms that provided a significant improvement in the overall fit of the model as measured by the likelihood ratio statistic (LRS χ^2 : $P \leq 0.05$).

Results

Incidence rate estimates

Of 509 premises contacted, 305 returned useable questionnaires (60% return rate) and, of these, 100 premises (33%) were defined as recurrent because they had at least one EGS incident prior to the questionnaire incident during the risk period (Table 2). The median EGS incidence rate and median numbers of horses were statistically significantly greater for recurrent than for nonrecurrent premises. Median EGS incidence rates adjusted for horse numbers on premises were consequently virtually identical, irrespective of whether EGS had recurred.

Univariable Poisson regression analyses

Tables 3 and 4 summarise numbers and proportions of recurrent and nonrecurrent premises for different categories of variables and show rate ratios, 95% confidence intervals and corresponding Wald χ^2 P values from univariable Poisson regression analyses.

Among horse- and premises-related variables (Table 3), there was a trend towards increasing rate of recurrence of EGS with increasing numbers of horses and stables on premises. Presence of

TABLE 5: 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 95% confidence intervals and corresponding Wald and LRS χ^2 P values

Variable	Category	Coefficient	Standard error	Rate ratio	95% CI		P value
					Lower	Upper	
<i>Intercept</i>		-2.57	0.28				
No. horses on the premises	1–5			Referent			<0.0001 [†]
	6–10	0.08	0.37	1.08	0.52	2.22	0.838
	11–15	1.06	0.32	2.90	1.55	5.42	0.001
	16–20	2.46	0.35	11.7	5.91	23.1	<0.001
	21–40	1.27	0.30	3.56	1.98	6.40	<0.001
	41+	1.97	0.31	7.18	3.92	13.2	<0.001
Presence of horses age <2 years	No			Referent			0.0001 [†]
	Yes [2YO]	0.53	0.24	1.70	1.06	2.71	0.027
Soil type	Clay			Referent			<0.0001 [†]
	Sand	0.36	0.20	1.43	0.98	2.10	0.067
	Chalk	-1.48	0.61	0.23	0.07	0.76	0.016
	Loam	0.74	0.21	2.11	1.40	3.16	<0.001
	Other	-0.98	0.44	0.38	0.16	0.90	0.027
Method of faeces removal [REM]	Not removed			Referent			<0.0001 [†]
	By hand [1]	-1.71	0.49	0.18	0.07	0.48	0.001
	Mechanically [2]	1.02	0.47	2.76	1.10	6.94	0.031
Pasture cut?	Not cut			Referent			<0.0001 [†]
	Cut [CUT]	-2.15	0.32	0.12	0.06	0.22	<0.001
Other domestic animals on the pasture [DOM]	None			Referent			<0.0001 [†]
	Ruminants [1]	-2.22	0.41	0.11	0.05	0.24	0.001
	Birds/fowl [2]	-0.09	0.30	0.91	0.50	1.65	0.760
	Other [3]	-2.37	1.03	0.09	0.01	0.70	0.021
[REM]*[2YO]	[REM 1]*[2YO]	1.22	0.50	3.40	1.27	9.11	0.015
	[REM 2]*[2YO]	-0.54	0.42	0.58	0.26	1.32	0.196
							0.004 [†]
[REM]*[CUT]	[REM 1]*[CUT]	1.22	0.46	3.39	1.37	8.38	0.008
	[REM 2]*[CUT]	0.26	0.40	1.30	0.59	2.84	0.515
							0.023 [†]
[DOM]*[CUT]	[DOM 1]*[CUT]	2.03	0.48	7.65	2.99	19.6	<0.001
	[DOM 2]*[CUT]	0.73	0.48	2.07	0.81	5.28	0.128
	[DOM 3]*[CUT]	2.91	1.06	18.3	2.29	147	0.006
							<0.0001 [†]

[†]Likelihood ratio statistic (LRS) χ^2 P value.

younger horses was also associated with an increased rate. Livery/riding establishments and stud farms had increased rates compared to farms.

For pasture management variables (Table 4), although there was an overall increased rate of recurrence associated with removal of faeces from pastures, when removal methods were specified, manual and mechanical methods had opposing directions of effect that were each statistically significant. Removal by hand was apparently protective, but mechanical removal was associated with a greater than 4-fold increase in rate of recurrence.

When reseeding of pastures, which was not in itself significantly associated with recurrence, was broken down by the period since reseeding was conducted, opposing directions of effect were identified. Reseeding within the last 5 years was associated with an increased rate of recurrence whereas reseeding more than 5 years previously was apparently protective. This variable and the related variable of ley pastures compared to permanent grazing, were considered likely effects of, rather than risk factors for, recurrence and were not, therefore, included in multivariable Poisson regression analyses. Similarly, as supplementary feeding of hay and concentrate to grazing horses had been advocated following studies by Gilmour and Jolly (1974),

the spring and summer supplementary feeding variables were also not included in multivariable modelling as they might have been a consequence of recurrence of EGS rather than contributing to it.

Multivariable Poisson regression analyses

Table 5 outlines the final multivariable Poisson regression model including several statistically significant interaction terms that were identified during model building, detailed in Table 6. 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 faeces removal. There was evidence for a decreased rate associated with chalk and other soil types, removal of faeces by hand, grass cutting and ruminants grazing pasture.

The effects of other domestic animals on pasture and methods of faeces removal from pasture were significantly modified according to whether pastures were cut and/or there were animals age <2 years present on premises. There was a lower rate associated with mechanical faeces removal on premises with animals age <2 years compared to those premises without such

TABLE 6: Details of significant effect modification between variables in the final multivariable Poisson regression model for recurrence of EGS on previously affected premises, with rate ratio interaction term estimates (underscored) applied to baseline rate ratios

Interaction between Variable 1*Variable 2		Variable 2	
Variable 1	Categories ▼	Categories ►	
<i>Method of faeces removal*presence of horses age <2 years</i>			
		Presence of horses age <2 years [2YO]	
		No	Yes
Method of faeces removal [REM]	None	Referent	1.70
	By hand [1]	0.18	0.18* <u>3.40</u> = 0.61
	Mechanically [2]	2.76	2.76* <u>0.58</u> = 1.60
		LRS χ^2 P value 0.004	
<i>Method of faeces removal*pasture cutting</i>			
		Pasture cutting? [CUT]	
		No	Yes
Method of faeces removal [REM]	None	Referent	0.12
	By hand [1]	0.18	0.18* <u>3.39</u> = 0.61
	Mechanically [2]	2.76	2.76* <u>1.30</u> = 3.59
		LRS χ^2 P value 0.023	
<i>Other domestic animals*pasture cutting</i>			
		Pasture cutting? [CUT]	
		No	Yes
Other domestic animals on the pasture [DOM]	None	Referent	0.12
	Ruminants [1]	0.11	0.11* <u>7.65</u> = 0.84
	Birds/fowl [2]	0.91	0.91* <u>2.07</u> = 1.88
	Other [3]	0.09	0.09* <u>18.3</u> = 1.65
		LRS χ^2 P value <0.0001	

LRS = Likelihood ratio statistic.

animals. The increased rate associated with mechanical removal increased further and the protective effect of manual removal was reduced when there was pasture cutting. The protective effect of grazing ruminants was apparent only when pastures were not cut. Similarly, there was an increased rate from having domesticated birds/fowl on premises, but only when pastures were cut.

Discussion

The aim of this study was to investigate risk factors for recurrence of EGS on previously affected premises and, as such, the study was designed to gather a wide range of information on the management of horses and pastures on these premises.

An area of potential bias in this study was the necessity to use owner reporting of cases and, consequently, reliance on using some cases diagnosed by clinical signs alone rather than having all cases confirmed histopathologically. Although the possibility of false positive diagnoses cannot be excluded, this was unlikely to be a systematic bias that would lead to serious errors in interpretation, as it was unlikely to be significantly associated with any of the risk factors being examined. In addition, with repeated occurrences of EGS on premises and familiarity with the disease, it is probable that clinical diagnosis becomes less inaccurate, thereby minimising misdiagnoses in the absence of histopathology. Another potential shortcoming of the study was that a measure of stocking density (i.e. numbers of animals per unit area of grazing) was not included in the analyses. However, although a stocking density measure was originally derived for each premises from the questionnaire data, it was soon recognised that the density measure derived was inappropriate for analysis purposes because it was based on the ratio of numbers of horses to the total area of the premises rather than only the area of available grazing. This would have significant consequences for stocking density estimates for

different types of premises, where not all of the area of the premises would be used for grazing horses (e.g. farms, Scottish estates). Rather than make inappropriate inferences relating to a potentially biased stocking density estimate, we chose to exclude this from our analyses.

The association of several factors with recurrence of disease was considered more likely to be an effect of EGS recurrence than a cause. Consequently, these factors were not investigated further by inclusion in multivariable analyses. These factors included the practice of supplementary feeding during the spring and summer, i.e. during the high risk period for EGS (Doxey *et al.* 1991b; Wood *et al.* 1998), which was suggested to be protective in an earlier study by Gilmour and Jolly (1974). It was also believed that reseeding of affected pastures might have been more likely to be performed on premises where the disease had recurred and particularly where this occurred recently, in an attempt to reduce future recurrence rather than being a factor contributing to it. This factor was also related to grazing type, as the majority of ley grazing had been reseeded in the previous 5 years.

The majority of variables retained in the final multivariable Poisson regression model demonstrated strong associations with recurrence of EGS on premises. We consider that knowledge of these factors may provide novel insights into the pathogenesis of EGS and consequently, although speculative, discussion of possible biological mechanisms to explain these findings is warranted. The identification of several significant interaction terms in the final multivariable model emphasises that considerable caution is required in interpreting findings of this study and that these observations ideally require specific hypotheses to be generated and subsequently tested in appropriately designed, controlled and randomised intervention studies.

Numbers of horses on premises was an important risk factor for recurrence of EGS. As it is horses and not premises that suffer the disease, it is logical that animals may each act as separate

sentinels for disease occurrence and, therefore, as numbers of horses increase, an increase in rate of recurrence would be expected. There was a similar trend in increasing rate of recurrence with increasing numbers of stables on premises, which was probably a proxy measure for numbers of horses. The importance of the number of horses on the premises was also illustrated, in that recurrent premises had an equivalent median EGS incidence rate to nonrecurrent premises when rates were adjusted for numbers of animals.

During multivariable modelling, the effects of different premises types became no longer significant. This suggested that there was confounding of premises type by the variables retained in the final model, particularly by number of horses and presence of younger animals. It could be shown that both these factors had considerable influence on the rate ratio for stud farms, which tended to have larger numbers of horses and particularly younger animals.

Results support earlier findings (Gilmour and Jolly 1974; Doxey *et al.* 1991b; Wood *et al.* 1998; McCarthy 2002; McCarthy *et al.* 2004) that younger horses are at increased risk of EGS compared to older animals, because after controlling for other significant factors there remained an overall increased rate of recurrence of EGS on premises on which horses age <2 years were present. There was again evidence for confounding of this factor by horse numbers on premises.

An apparently novel finding in this study was that the rate of recurrence of EGS varied significantly between premises on different soil types. Results of multivariable analysis showed that premises on loam and sand soils had increased rates of recurrence compared with premises on clay, whereas premises on chalk and other soil types had significantly reduced rates of recurrence. Sand and loam soils are acidic, light and well-draining. Chalk soils are relatively shallow and compacted and very different types of plants grow in them. Clay soils provide good supplies of plant nutrients, but have high water-holding capacity, which fills air spaces within the soil and makes them very heavy. Variation in rates of recurrence of EGS on premises of different soil types may therefore be consistent with the theory that the disease is due to toxico-infectious form of botulism (Tocher *et al.* 1923; Tocher 1924; Hunter *et al.* 1999; Hunter and Poxton 2001; McCarthy 2002; McCarthy *et al.* 2004). *C. botulinum* resides in the soil and characteristics of sand and loam soils may permit these soil types to be more easily disturbed and turned-over than clay soils. Therefore, soil inhabitants, such as earthworms and moles, are able to burrow more freely through sand and loam soil types, causing disruption and increasing the rate of soil contamination of grass and hence bringing the bacterium into contact with grazing horses more frequently than with clay, chalk and other soils. The characteristics of chalk soils, which are shallow and impacted, tend to limit the activity of soil inhabitants compared to other soil types. Soil type as a significant risk factor for recurrence of EGS on premises is consistent with some findings by McCarthy (2002) that disturbance of pasture, such as by moles or excavation, was associated with an increased risk of disease. This hypothesis requires further investigation to determine the validity of the complimentary findings of these 2 independent studies. If soil type and soil disturbance are truly associated with risk of recurrence, they may help to explain geographical differences in occurrence of EGS and need to be considered in future predictions of disease occurrence for specific premises or affected premises within particular areas of the UK. It would be particularly interesting to quantify *C. botulinum* at different depths in specific soil types in

areas in which EGS does and does not occur and to assess the extent of contamination of herbage by these bacteria under different management conditions.

Mechanical faeces removal was identified as an important risk factor for recurrence of EGS. This was expected to be a proxy measure for the numbers of horses on premises and for the premises type, as stud farms and other larger establishments with many horses frequently remove faeces mechanically, usually using paddock sweepers. Therefore, the association between the method of faeces removal and recurrence of EGS was expected to disappear in multivariable analyses after controlling for these other factors. Although there was some evidence for confounding in multivariable analyses (because the odds ratios were reduced from those in univariable analyses), there remained a significantly increased rate of EGS recurrence associated with mechanical removal, and removing faeces from pastures by hand provided evidence of a protective effect. In addition, the association with faeces removal method was modified according to whether there were younger horses or grass cutting on premises. The mechanism by which mechanical removal increased the rate of recurrence of EGS compared to the protective effect of manual removal is unclear. However, an aetiological agent or factor in faeces or soil may be important and the process of sweeping paddocks mechanically may disseminate this agent at the same time as disturbing the soil surface. Both factors may contribute to dissemination of faecal material and soil that contain clostridia on to herbage, thereby bringing grazing horses into contact with them. Manual removal may avoid soil exposure to horses by discouraging overgrazing of pastures and reducing exposure to faecal material. The hypothesis relating to methods of faecal removal further supports the views of Begg (1936) and Wood *et al.* (1999) that the aetiological agent is present in the soil rather than the grass and would be consistent with EGS being a toxico-infectious form of botulism.

Having other domestic animals on premises with horses was associated significantly with variations in the rate of recurrence of EGS, although there was significant interaction with pasture cutting, which make interpretation of these factors difficult at this stage. There was evidence that grazing ruminants conveyed some protection against recurrence, although it is unclear whether this is simply attributable to consequently reduced grazing intensity by horses or to some indirect mechanism related to removal of toxic material, bacteria or parasites from the pasture. There was also an increased rate of recurrence associated with the presence of domesticated birds or fowl on premises. This finding corresponds with anecdotal observations that recurrence of EGS frequently occurred in areas where intensive rearing of game birds occurred. In addition, birds have long been recognised as a source of botulism and the importation of 'guano' (a nitrogenous fertiliser of avian origin) from South America in the mid/late 19th century to improve Scottish agricultural land was soon followed by the first reports of EGS in this part of Britain (K. Miller, personal communication). However, as birds and fowl occur worldwide and these animals have been linked with classical botulism, but not EGS, in many areas, further studies of important cofactors and distributions of specific botulinum toxin types are warranted.

Grass cutting on pastures was associated with a significantly reduced rate of recurrence of the disease, although there were apparently significant interactions with faeces removal methods and other domestic animals on pastures. This protective association may again have been attributable to reduced grazing intensity by horses but may also have encouraged more even

grazing of pastures and hence reduced overgrazing of specific areas, which might be more likely to expose soil and bring horses in contact with soil-borne bacteria.

Although access to grazing has historically been the major risk factor for EGS (Begg 1936; Gilmour and Jolly 1974), 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 (Wood *et al.* 1999). The risk factors identified in this study are consistent with the hypothesis that EGS is caused by toxico-infection by *C. botulinum*. As a result of these findings, it is very tempting to suggest possible protective control measures for premises affected with EGS, such as good pasture management, cograzing of ruminants and the avoidance of pasture sweepers and domesticated birds. However, these interventions cannot be entirely justified from this single, retrospective study. 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.

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References

- Araya, O., Vits, L., Paredes, E. and Ildefonso, R. (2002) Grass sickness in horses in southern Chile. *Vet. Rec.* **150**, 695-697.
- Barlow, R.M. (1969) Neuropathological observations in grass sickness of horses. *J. Comp. Path.* **79**, 407-411.
- Begg, G.W. (1936) Grass sickness in horses. *Vet. Rec.* **48**, 655-662.
- Doxey, D.L., Milne, E.M., Gilmour, J.S. and Pogson, D.M. (1991a) Clinical and biochemical features of grass sickness (equine dysautonomia). *Equine vet. J.* **23**, 360-364.
- Doxey, D.L., Gilmour, J.S. and Milne, E.M. (1991b) A comparative study of normal equine populations and those with grass sickness (dysautonomia) in eastern Scotland. *Equine vet. J.* **23**, 365-369.
- Doxey, D.L., Milne, E.M. and Harter, A. (1995) Recovery of horses from dysautonomia (grass sickness). *Vet. Rec.* **137**, 585-588.
- Doxey, D.L., Johnston, P., Hahn, C. and Reynolds, J. (2000) Histology in recovered cases of grass sickness. *Vet. Rec.* **146**, 645-646.
- Frome, E.L. (1983) The analysis of rates using Poisson regression models. *Biometrics* **39**, 665-674.
- Gilmour, J.S. and Jolly, G.M. (1974) Some aspects of the epidemiology of equine grass sickness. *Vet. Rec.* **95**, 77-81.
- Greig, J.R. (1942) Grass sickness in horses: a review of the present knowledge of the disease, with particular reference to the nature of the causal agent. *Trans. Highland Agric. Soc. Scotland* **54**, 1-27.
- Hosmer, D.W. and Lemeshow, S. (1989) *Applied Logistic Regression*, John Wiley, New York.
- Hunter, L.C. and Poxton, I.R. (2001) Systemic antibodies to *Clostridium botulinum* type C: do they protect horses from grass sickness (dysautonomia)? *Equine vet. J.* **33**, 547-553.
- Hunter, L.C., Miller, J.K. and Poxton, I.R. (1999) The association of *Clostridium botulinum* type C with equine grass sickness: a toxicoinfection? *Equine vet. J.* **31**, 492-499.
- John, H.A., Creighton, A.J. and Baird, A. (2001) Thoracic sympathetic chain ganglion neuronal abnormalities that may explain some of the clinical signs of grass sickness. *Vet. Rec.* **148**, 180-182.
- Mahaffey, L.W. (1959) Ganglionic lesions in grass sickness of horses. *Vet. Rec.* **71**, 170-171.
- McCarthy, H.E. (2002) *A Case Control Study to Investigate Risk Factors for Equine Grass Sickness with a Particular Reference to the role of Clostridium botulinum*. PhD Thesis, University of Liverpool.
- McCarthy, H.E., Proudman, C.J. and French, N.P. (2001) Epidemiology of equine grass sickness: a literature review (1909-1999) *Vet. Rec.* **149**, 293-300.
- McCarthy, H.E., French, N.P., Edwards, G.B., Poxton, I.R., Kelly, D.F., Miller, K. and Proudman, C.J. (2004) Equine grass sickness is associated with *Clostridium botulinum*. Findings from a matched case-control study to identify risk factors. *Equine vet. J.* **36**, 123-129.
- Milne, E.M. (1996) Clinical diagnosis and management of acute and subacute grass sickness. *Equine vet. Educ.* **8**, 71-73.
- Obel, A.L. (1955) Studies on grass disease: the morphological picture with special reference to the vegetative nervous system. *J. Comp. Pathol.* **65**, 334-354.
- Scholes, S.F.E., Vaillant, C., Peacock, P., Edwards, G.B. and Kelly, D.F. (1993) Enteric neuropathy in horses with grass sickness. *Vet. Rec.* **132**, 647-651.
- Tocher, J.F. (1924) Grass sickness in horses. *Trans. Royal Highland Agric. Soc. Scotland* **36**, 65-83.
- Tocher, J.F., Brown, W., Tocher, J.W. and Buxton, J.B. (1923) 'Grass sickness' investigation report. *Vet. Rec.* **3**, 37-45; 75-89.
- Uzal, F.A. and Robles, C.A. (1993) *Mal seco*, a grass sickness-like syndrome of horses in Argentina. *Vet. Res. Comm.* **17**, 449-457.
- Wood, J.L.N., Milne, E.M. and Doxey, D.L. (1998) A case control study of grass sickness (equine dysautonomia) in the United Kingdom. *Vet. J.* **156**, 7-14.
- Wood, J.L.N., McGorum, B.C. and Mayhew, I.G. (1999) Equine dysautonomia: has grass been blamed unfairly all this time? *Equine vet. J.* **31**, 451-452.

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