



Science: Overviews

Equine grass sickness: Are we any nearer to answers on cause and prevention after a century of research?

Introduction

Equine grass sickness (EGS) remains a debilitating and frequently fatal neurodegenerative disease of horses. Although the disease was first recognised in the early 1900s, the definitive cause still remains to be elucidated. A peer-reviewed survey by Mellor *et al.* (2001) ranked EGS as the most important disease of horses within Scotland and northern England, suggesting that research priorities should focus on identifying a cause and ideally a prevention for this significant disease. This scientific overview reviews the main developments in EGS research in the past 100 years since the disease was first recognised, as they relate specifically to determining the cause of EGS, and concludes by considering a proposed field trial of a preventive *Clostridium botulinum* (*C. botulinum*) toxoid that is currently being developed by the authors.

In this issue of Equine Veterinary Journal (pp 494-499), Waggett *et al.* (2010) investigate the prevalence of *Clostridium perfringens* in faeces and ileal contents from grass sickness.

Epidemiology of EGS

The first case of EGS occurred in 1909 in eastern Scotland (Tocher *et al.* 1923) with subsequent confirmation in other regions of the UK and parts of northern Europe (Woods and Gilmour 1991; McCarthy *et al.* 2001; Wylie and Proudman 2009). The condition 'mal seco' (literally translating as 'dry sickness') is regularly diagnosed in regions of South America and, following comparative work, is now regarded as the same disease as EGS (Uzal and Robles 1993; Araya *et al.* 2002). A recently published case report describes a 6-year-old mule in North America with clinical signs and a histological diagnosis consistent with EGS. While EGS has previously been suspected in the USA, this is the first published report describing the disease in a clinical case (Wright *et al.* 2010).

The first epidemiological survey of EGS was carried out in Scotland in the 1970s by Gilmour and Jolly (1974), and a UK-wide matched case-control study was conducted by Wood *et al.* (1998). As the name suggests, there is a strong association between the development of EGS and access to grazing, with grazing animals accounting for >99% of cases. Indeed, only 2 case reports, each almost 70 and 50 years old, respectively, have been published reporting EGS in nongrazing horses (Forsyth 1941; Lannek *et al.* 1961). A number of studies have confirmed that EGS has a strong seasonal distribution (Doxey *et al.* 1991; Wood *et al.* 1998; French *et al.* 2005; Archer *et al.* 2006). The peak season is the spring, with May consistently producing the greatest number of cases in the UK (Fig 1). Cases have been identified in all months of the year, with a second smaller peak reported in the autumn (Doxey *et al.* 1991;

Archer *et al.* 2006). There is an increased risk of disease associated with previously affected premises (with the risk decreasing over time) and recent changes of pasture (Gilmour and Jolly 1974; Wood *et al.* 1998; McCarthy *et al.* 2004a). Wood *et al.* (1998) also found a 10-fold reduction in risk among horses in contact with previous cases, consistent with the acquisition of immunity and/or resistance to disease. A study of EGS cases conducted in England and Wales reported evidence of significant spatial and temporal clustering emphasising the recurrence of disease on previously affected premises (McCarthy *et al.* 2004a). This is entirely consistent with anecdotal reports and clinical experience of the disease.

Newton *et al.* (2004) conducted an observational study to identify risk factors for recurrence of EGS on previously affected premises. There was evidence for an increased rate of recurrence with higher numbers of horses, presence of younger animals and domestic birds, loam and sand soils, studs and livery/riding establishments and mechanical droppings removal. The rate of recurrence apparently decreased with the presence of co-grazing ruminants, chalk soil, pasture grass cutting and manual dropping removal (Newton *et al.* 2004). Despite this research there are currently no scientifically validated methods to prevent EGS in susceptible animals.

Botulism as a cause

Over the past 100 years a number of different aetiologies have been proposed including mineral and vitamin deficiencies, toxic plants, insects, fungi, filterable viruses and bacterial toxins. Among these, there is both historical (1920s) and modern (1990s–present) scientific evidence to support the hypothesis that EGS is a toxicoinfectious form of botulism involving *C. botulinum* type C toxins: the C1 neurotoxin (BoNT/C) and possibly the C2 binary toxin produced locally within the gastrointestinal tract (Tocher *et al.* 1923; Poxton *et al.* 1997; Hunter *et al.* 1999; Hunter and Poxton 2001; Hedderson and Newton 2004; McCarthy *et al.* 2004b). Tocher *et al.* (1923) first proposed the botulinum hypothesis in 1919 following his observations that muscle tremors, dysphagia, ptosis, salivation and a tucked-up abdomen were clinical signs consistent with both botulism and EGS, gross *post mortem* examinations, which suggested acute toxæmia of bacterial origin (Tocher *et al.* 1923), and isolation of a 'large anaerobic bacillus', which possessed similar morphological characteristics and toxigenic properties of *C. botulinum*.

In 1922 and 1923 controlled vaccine trials were performed involving more than 2000 horses, with half the horses on each premises receiving an antitoxin neutralised botulinum toxin vaccine and the other half remaining as unvaccinated controls (Tocher *et al.* 1923; Wood *et al.* 1999; Collier *et al.* 2001). Results in both years of

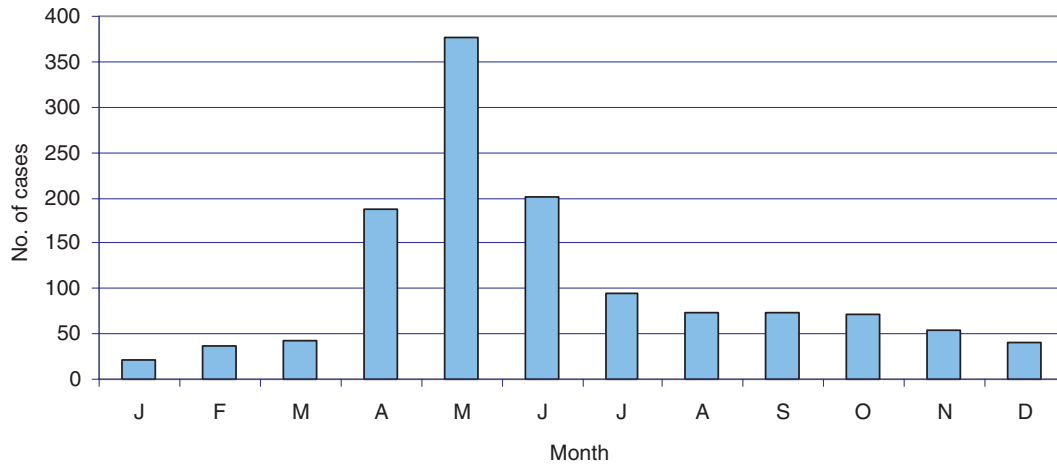


Fig 1: Distribution by month of occurrence of EGS cases in GB between 2000 and 2009 (n = 1274). Data collated by the AHT-based equine grass sickness surveillance scheme.

TABLE 1: Summary of results from botulinum vaccine trials, conducted by J.F. Tocher in Scotland in 1922 and 1923 to prevent EGS. Results show evidence of highly statistically significant protective effects and possible dose-response effect in both trials

Year of trial	Vaccine doses (n)	Fatal EGS cases (n)	Total horses (n)	Prevalence (%)	Odds ratio
1922	0	33	355	9.3%	1.00 (Ref.)
	1	18	566	3.2%	0.32
	2	9	395	2.3%	0.23
	Total	60	1256	4.8%	*P = 0.00001
1923	0	37	453	8.2%	1.00 (Ref.)
	1 or 2	7	472	1.5%	0.17
	2	0	136	0%	0.08#
	Total	44	1062	4.1%	*P<0.00001

Ref. = Referent category. *Chi-squared test for trend. #Analysis based on creation of one artificial fatal EGS case for the 2 doses group.

the study showed a highly statistically significant reduction in EGS mortality rate among the vaccinated group with strong evidence of a dose-response effect ($P < 0.000001$) (Table 1). In 1922, EGS mortality among nonvaccinated horses was 9.3% compared with 3.2% among those that had been inoculated once with human vaccine and 2.3% for those that received 2 doses of human vaccine. A more potent vaccine was used in the subsequent trial in 1923 this time utilising *C. botulinum* isolated from chronic EGS cases with 8.2% mortality among nonvaccinated horses and a 1.5% mortality in horses vaccinated once. No horses that received 2 doses of vaccine died in the 1923 trial. Despite these results the botulinism theory for EGS was subsequently abandoned after Professor Gaiger from the University of Liverpool, who had his own streptococcal theory as to the cause of EGS, publicly criticised the trial.

Botulism revisited

Tocher's botulinum hypothesis was revisited in the early 1990s by Jean Robb and Keith Miller with further investigations conducted into the hypothesis that EGS is a toxico-infection with *C. botulinum* type C. In the theory of EGS being caused by a toxico-infectious form of botulism, toxin production (BoNT/C and C2 binary toxin) and absorption occurs mainly in the ileum due to overgrowth from normal large intestinal flora and/or due to spore germination in association with potential dietary trigger factors.

Further work has identified a significant association between the presence of both *C. botulinum* and BoNT/C in the ileal contents and faeces of histologically confirmed EGS cases compared with

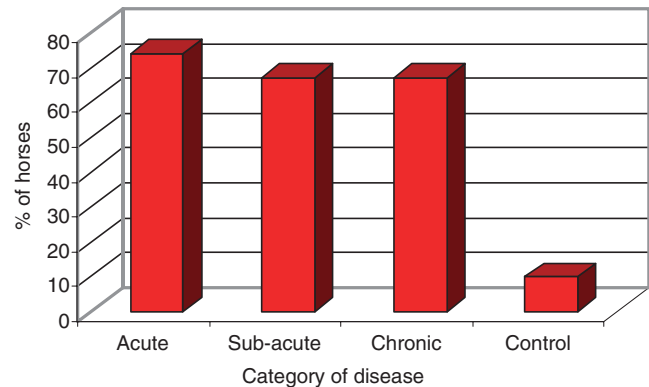


Fig 2: Proportion of horses from which *C. botulinum* neurotoxin C (BoNT/C) was identified in ileal contents and/or faeces by direct detection and/or enrichment, varied markedly between all types of histologically confirmed EGS cases and nonaffected controls (data from Hunter *et al.* 1999).

apparently healthy controls (Hunter *et al.* 1999) (Fig 2). These workers also found that EGS cases had significantly lower IgG antibodies to surface antigens of *C. botulinum* and BoNT/C than co-grazing horses that had either grazed the premises, or been in contact with a case (Hunter and Poxton 2001). A matched case-control study involving 66 histologically confirmed EGS cases and 132 matched controls further identified that, after accounting for other risk factors and confounders, the risk of EGS decreased

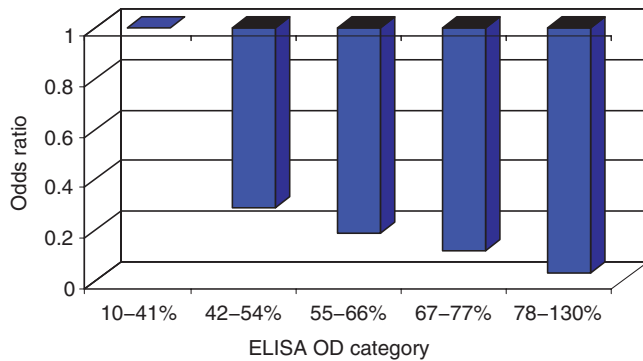


Fig 3: Risk of EGS after controlling for age decreases along a biological gradient with increasing systemic IgG antibodies to *C. botulinum* type-C (data from McCarthy *et al.* 2004b).

with increasing levels of systemic IgG antibodies to *C. botulinum* type C, *C. novyi* type A surface antigens and BoNT/C (McCarthy *et al.* 2004b) (Fig 3). The importance of other factors in the disease pathogenesis was highlighted by a recent finding in Israel, where EGS has never been reported but 31% of horses were seropositive to BoNT/C (Steinman *et al.* 2007). Nunn *et al.* (2007a) demonstrated an increased level of systemic IgG against the surface antigen of *C. botulinum* in surviving chronic cases compared to nonsurviving chronic cases suggesting that antibody status may not only influence the development of the disease but also the likelihood of survival (Nunn *et al.* 2007a). The same group also found that EGS cases had a significantly higher small intestine IgA (mucosal) antibody level against *C. botulinum*, and against BoNT/C and D compared to controls postulated to be indicative of recent exposure (Nunn *et al.* 2007b).

These findings support the likely protective role of anti-botulinum type C toxin antibodies in conveying immunity against EGS. If this hypothesis is correct then probably the best and easiest means of protecting large numbers of horses from EGS will be through effective and safe vaccines, which currently do not exist (Hedderson and Newton 2004). This approach has proved easy and successful in protecting horses, one of the most susceptible species, against the fatal effects of tetanus toxicosis, another clostridial disease.

***Clostridium botulinum* as the cause of dysautonomias in other species**

Dysautonomias occur in many animal species, but their aetiologies are largely unknown. However, dysautonomias in leporids (hares and rabbits) have pathological features similar to EGS (Whitwell 1991; Whitwell and Needham 1996) and the currently uncommon feline dysautonomia, also known as Key-Gaskell syndrome, is probably identical to the disease occurring in horses and leporids. An outbreak of feline dysautonomia in a colony of 8 cats, 6 of which showed clinical signs of the disease, was investigated for the involvement of *C. botulinum*. BoNT/C was detected in all 6 cases and one unaffected colony-mate, but was absent from 11 healthy controls. BoNT/C was also found after enrichment in a sample of the dried cat food. Serology demonstrated exposure to *C. botulinum* in the affected cats as a statistically significantly higher level of IgA antibody to BoNT/C and cell surface antigens were found in their faeces compared to that detected in the healthy controls (Nunn *et al.* 2004).

Future botulism vaccination to prevent EGS

As there is currently no reproducible animal model of EGS in which to demonstrate Koch's postulates for causation of EGS by toxico-infectious botulism, the best alternative for testing the hypothesis is through an appropriately conducted clinical field trial of a botulinum toxoid vaccine (Hedderson and Newton 2004).

In 2008, Bransby Home of Rest for Horses funded work at the Animal Health Trust to clone and express inactivated toxins, or fragments thereof, and test the safety in mice and horses. Subsequently a traditional, formaldehyde inactivated botulinum type C toxoid vaccine (Febrivac BOT) marketed for use in mink became available through a commercial collaborator which has been shown to be safe in mice and guinea pigs and is efficacious in mink. Both the toxin fragments and the commercially available type C toxoid vaccine may be used safely in horses and there was preliminary evidence that immune responses are generated against both types of vaccine (C. Robinson, personal communication). Unfortunately, the recombinant toxoid fragments produced at the AHT were very difficult to work with and are not considered suitable to be taken forward as vaccine candidates. However, the Febrivac BOT vaccine is suitable to be taken forward as it appears to be safe in horses and due to its method of production is likely to produce a broad ranging immunity against *C. botulinum* as well as its secreted toxins. Additional experiments are being undertaken to validate Febrivac BOT further as a vaccine candidate for the prevention of EGS. One approach will be to show that the immunological responses produced in vaccinated ponies are protective against toxic challenge using a mouse botulinum toxin challenge model, i.e. that post-vaccination pony serum neutralises toxin administered to mice. Another approach will be to demonstrate equivalence between antibody levels following vaccination and the higher levels quantified in presumptively immune control horses studied by McCarthy *et al.* (2004b). If it is demonstrated that there was a protective and/or immunological effect then this should help facilitate appropriate licensing of Febrivac BOT as an experimental vaccine for horses by the Veterinary Medicines Directorate for a randomised field trial against EGS.

A future UK-based EGS vaccine field trial would need to be multi-centred, randomised, controlled and blinded. A required sample size has been calculated based on assumptions of: 1) relative risk (RR) of 0.25 (i.e. the vaccine would provide at least a 4-fold reduction in risk of EGS); 2) an average disease rate in controls of 2 cases/100 horse/year (based on data from Newton *et al.* 2004); 3) 5% significance level; 4) 80% power; and 5) up to 10% loss of study horses to follow-up. Using these assumptions, the required sample size was calculated as approximately 1800 horse-years of follow-up (Hedderson and Newton 2004).

National surveillance of EGS

A multicentre collaborative project involving the Animal Health Trust, Newmarket, The Universities of Edinburgh and Liverpool and the Equine Grass Sickness Fund for the surveillance of EGS in the UK, and initially funded by The Horse Trust, was initiated in 2007. The ongoing project (<http://www.equinegrasssickness.co.uk>) collates details of clinical cases occurring prospectively within GB and gathers retrospective information about cases occurring since 2000 on affected premises to investigate changes in the distribution and frequency of the disease. Further interrogation of the data held

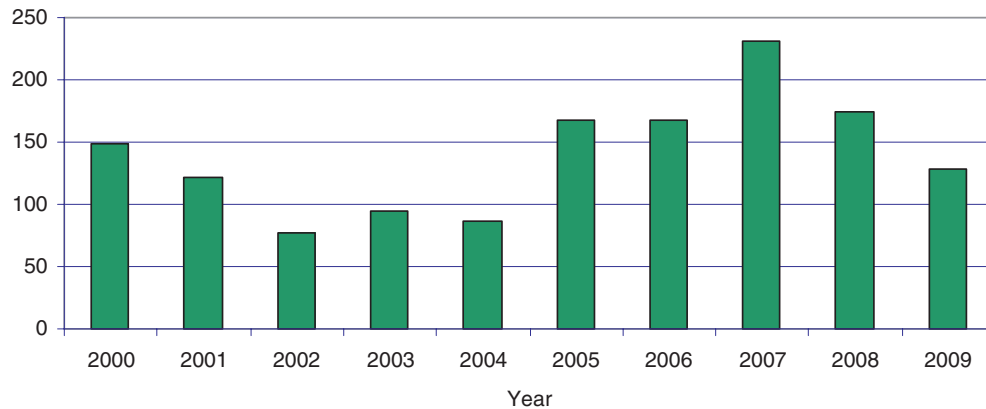


Fig 4: Distribution by year of occurrence of EGS cases occurring in GB between 2000 and 2009 ($n = 1398$). Data collated by the AHT-based equine grass sickness surveillance scheme.

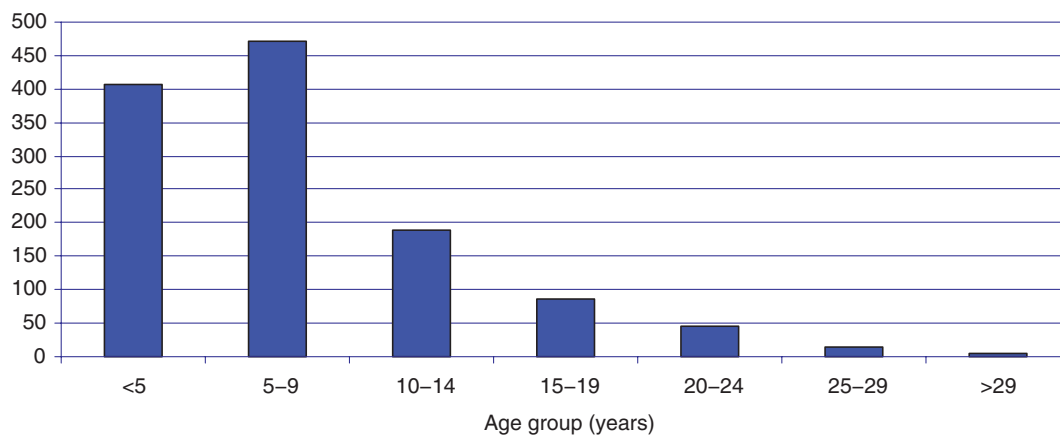


Fig 5: Distribution by age in years of EGS cases occurring in GB between 2000 and 2009 ($n = 1219$). Data collated by the AHT-based equine grass sickness surveillance scheme.

within the database may identify premises deemed to be 'high-risk' within the last decade and these owners may be specifically approached to be involved in a future randomised, controlled field trial.

As of January 2010, the database held details of 2572 EGS cases occurring in GB between the years 1942 and 2009, and included details from 1613 individual owners. Among these cases, 1398 occurred since the beginning of the year 2000, with numbers of annually recorded cases appearing to vary in a sinusoidal pattern (Fig 4). Consistent with previous studies, most cases of EGS reported to the scheme occur in the spring, especially May which accounts for 29.6% of cases (Fig 1).

Among the cases since 2000, 34% were of the chronic form, 45% were acute and the remainder (21%) sub-acute. The owner-reported prevalence of survival of selected chronic grass sickness cases during this 10 year period was 49%. The age range of the cases reported was 2 months to 47 years with a mean of 7.5 years, median of 6 years and mode of 5 years. When only horses aged ≤ 9 years considered ($n = 878$), the mean and median age of horses with EGS is lower at 5 years, highlighting a long tail to the data occupied by fewer animals (Fig 5).

Among horses affected since 2000, 46% were mares, 48% were geldings and 6% were stallions, with no statistically significant differences observed in terms of the form of EGS diagnosed (acute, sub-acute or chronic) between the genders. A wide representation

of breeds are listed as affected on the database with cross-breeds represented most frequently, accounting for 34% of all cases. Sixty-six different pure breeds are listed, of which Thoroughbreds (12%), Highlands (9%) and Welsh Cobs (8%) are most commonly represented. At present, lack of validated denominator data for the UK preclude meaningful analyses to determine whether the gender and breed distributions represent any truly significant alteration in risk to EGS. The continued surveillance of EGS in GB should facilitate subsequent field evaluation of any effect of intervention such as botulinum vaccination in reducing the frequency of occurrence of the disease.

Conclusions

The beginning of the second decade of this millennium coincides with the first century since the first cases of EGS were described and heralds an important phase of grass sickness research. It is hoped that future research and surveillance will provide a definitive answer to the question that has not been adequately answered over the past 100 years as to the true cause of the disease, and from this should inform the most appropriate methods for preventing it. The authors hope that their current collaborative approach and endeavours in trying to establish a botulinum toxoid vaccine trial will ultimately bring long lasting benefits for horse welfare in Britain and further afield. If this is achieved it will provide a stark

contrast to the stifling effect that the professional rivalry between Tocher and Gaiger had on facilitating EGS prevention through vaccination many decades previously.

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