

## Grass sickness - the same old suspects but still no convictions!

Our search for the aetiological culprit of grass sickness (equine dysautonomia) has all the ingredients of an Agatha Christie whodunnit. The first bodies were discovered in a military camp in Scotland in 1909 (Tocher 1924). Was the killer of recent birth or had the culprit lain dormant or unidentified up to that time? Had the equine victims recently returned from abroad, importing some agent from another location? Equine grass sickness (EGS) is now recognised in England, Scandinavia and Switzerland, in the Falkland Islands and Patagonia. In a strange twist, the condition occurs rarely in North America, Australia or Ireland.

The earliest formal research into EGS was reported to the Trans Highland Agricultural Society of Scotland (Tocher *et al.* 1923), after exhaustive field investigations by Dr Tocher and his collaborators. By that time the disease had spread slowly to parts of England, although Eastern Scotland continued to suffer the greatest number of deaths, 10–15% of the horses in many areas. Various epidemiological factors were identified; most at risk were horses first put out to grass in the spring, particularly if there had been cold weather and prolonged drought. Several possible culprits were eliminated by researchers in the 1920s and *C. botulinum* (then called *Bacillus botulinus*) rose to the top of the list. It had been isolated from spleens of EGS cases, and toxin from the organism was found to produce signs of acute EGS when injected subcutaneously (Tocher *et al.* 1923). Vaccination trials (Tocher 1924), utilising a toxin/antitoxin mixture, derived from known strains of *botulinus* (probably type B) involving over 2000 horses were performed as randomised control trials (half the horses on each farm were inoculated and the remaining animals acted as controls, methodology currently considered as the gold standard). In the first year of the study, 2 doses of the toxin/antitoxin mixture were given in the spring; this resulted in a 2.8% mortality rate in inoculated, compared to 9.3% in uninoculated control animals. Using vaccine with a higher protective value in the second year, the mortality of uninoculated, animals was again about 10%, compared to only about 1.5% of inoculated horses.

Dr Tocher's work was vociferously and, it now seems, wrongly, criticised by Professor Gaiger, a veterinary pathologist. He offered his own explanation that *streptococci* were to blame and, further, claimed that Tocher's vaccine was causing 'blind staggers' in some horses (this latter without any specific data). In 1924, Gaiger was appointed Chief Investigator for the Animal Diseases Research Association and convinced his colleagues to give his organisation the EGS research remit. Unfortunately, no findings had been reported as late as 1930; and we are

unaware of Gaiger's findings ever being formally reported.

In true Agatha Christie style, mystery clouds the next 60 years when Tocher's promising detective work became largely ignored by researchers. Why might the trail have gone cold, despite the commercial availability of an EGS vaccine (Fig 1)? Firstly, the new lead researcher was adamantly opposed to the theory that *Bacillus botulinus* was to blame. Secondly, subsequent researchers did not appreciate the essential distinction between a botulinum 'toxicoinfection' and the classic primary intoxication of toxin-induced botulism (see, for example, McCarthy 2001 citing a 1929 experiment wherein botulinus toxin was stomach-tubed into ponies; the ponies - unsurprisingly - developed classic botulism signs and not those of EGS; this experiment with the toxin only was hailed, at the time, as proof that there was no relation between *C. botulinus* and EGS). Tocher himself understood the distinction between toxicoinfection by the organism vs. classic botulism, warning: "It should be clearly understood that, in all cases of grass sickness, horses are first infected with the *Bacillus botulinus* which in turn produces

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its deadly toxin in the tissues, finally causing the death of the horse.” He further stated: “Any normal animal can swallow a large number of these spores without any ill effect, the spores being passed from the gut of the animal without doing any harm. There must be a predisposing cause to EGS”; he thought it likely to be gastric irritation. Finally, in a double blow to equine researchers generally, the Great Depression arrived and tractors became readily available making horses less essential to agriculture. Research funds dried up.

So what further clues have been reported since the 1920s? Current geographical distribution seems to share similar climatic variations associated with EGS; cool dry weather with ground frost (Doxey *et al.* 1991a). Wood *et al.* (1998) noted that 66% of cases occurred following a 2 week period of predominantly dry weather, followed by rain. Could these climatic conditions allow the development of some toxin on pastures? One candidate is *Fusarium graminearium*, a mycotoxin-producing fungus that has been commonly isolated from paddocks where confirmed cases have occurred (Doxey 1991a; Robb 1997). Uzal *et al.* (1997) fed *Fusarium* cultures to 4 horses for 4 days; the animals showed signs of mild to moderate colic. Furthermore, *Fusarium graminearium* mycotoxin is a severe gastrointestinal irritant and is immunosuppressive; it probably has its greatest effect on gut-associated lymphoid tissue (Pestka *et al.* 1987), thereby compromising mucosal immunity. In an interesting parallel, mycotoxin production goes into overdrive at near freezing temperatures and a dry substrate would further stress the organism into producing even larger amounts of its toxins. Could this fungus be Tocher’s ‘predisposing factor’, the primary insult which then allows *C. botulinum* to invade the gut tissues and proliferate its deadly toxin?

Relevant clues unrelated to environmental factors include the use of anthelmintic drugs, possibly changing the natural flora of the gut (Wood *et al.* 1997). Age-related occurrence may provide another hint, as EGS particularly affects young horses (age 3–5 years) but only rarely foals (Doxey 1991b). Does the development of mature gut anatomy (Smyth 1988; Frappe 1998) and function (Smyth *et al.* 1989), achieved by age 4 months, play a role in pathogenesis, thereby providing a further clue for investigating workers? Certainly the pH of an immature GI tract may influence clostridial growth and toxin production (D. Lucas, personal communication) as the pH of the foal’s stomach is lower than that in older horses (Murray and Grodinsky 1989). Further, clostridial development in ensiled crops may occur in microniches where optimum water activity, pH and other conditions are created by yeasts and *Bacillus* spp. (McDonald *et al.* 1991). It is possible that suitable microniches also occur in the equine gut. Generalised stress factors may also be implicated; Doxey *et al.* (1991b) reported that 45% of the EGS cases investigated had been moved from one premises to another, probably involving a change in diet, and a further 12% had been stressed by events such as castration, foaling, breaking in or experiencing an accident within 2 weeks of illness. In another twist, animals grazing on paddocks where EGS had occurred were more likely to develop the disease but, if they had been in actual contact with a case, they were 10 times less likely to develop EGS (Wood *et al.* 1997). How can these unusual findings be accommodated? Perhaps immunological factors are involved; foals may have passive

immunity to EGS which diminishes by early maturity (longitudinal antibody assay would answer this hypothesis); active immunity, related to antigenic exposure and subsequent immune response, then kicks in at full maturity. This analysis would explain why horses in contact with EGS cases are less likely to develop the condition, having mounted an immunological response to the causative agent.

Other species develop a similar condition. Whitwell (1991) reported that 2 hares who died on a farm where 2 mares had died of EGS showed alimentary tract changes and polygangliopathy remarkably similar to those in EGS. A similar condition also occurs in cats (Key and Gaskell 1982; Whitwell 1997). A rare human dysautonomia occurs due to an anti-ganglioside antibody arising in response to infection with *Campylobacter* (A. Compston, personal communication). If anti-gangliosid antibodies damage autonomic ganglia in horses, this could lead to gut stasis and secondary overgrowth by *C. botulinum*. There is also a rare infant ‘botulism’ which is, in fact, a toxicoinfection by *C. botulinum* (Palin and Brown 1979).

What do current researchers now consider to be the prime suspect as causative agent? If ongoing and recently reported research is any indication, *Clostridium botulinum* is once again top of the ‘most wanted’ list. Hunter *et al.* (1999) demonstrated the presence of one of the toxins produced by *C. botulinum* type C in the faeces of 44% of EGS cases compared to 4% in controls (low sensitivity of the assay used in this work was reported as the reason that the toxin was not found in all cases of EGS). In this issue (p 547), Hunter and Poxton (2001) report their results of the measurements of antibody levels to *C. botulinum* type C (actually, for safety, *Clostridium novyi* type A, phenotypically similar to *C. botulinum* type C and immunologically cross-reactive, was used). Although it should be noted that their data range was wide and the mean/median difference small, they found the only significant difference in antibody levels was between cases of acute, subacute and chronic grass sickness (low levels) compared to contact/high risk animals (high levels); there was no significant difference found when each of these 2 groups was compared to controls. Odd, that. Could this result reflect failure by the EGS horses to mount an immune response, while the high risk group derived protection from their raised antibody levels? Perhaps circulating antibody in the affected individuals was consumed by excess toxin? Or is *C. botulinum* merely opportunistic and are we simply seeing a secondary infection by the organism due to gut stasis? More focused work in this area would certainly seem fruitful.

An experimental ELISA assay for measuring blood levels of *C. botulinum* type C toxin in wild birds has been developed (Rocke *et al.* 1998) and this could be used to measure directly the toxin levels in cases with grass sickness. Furthermore, specific detection of *C. botulinum* type C organisms can be achieved in tissue samples using the polymerase chain reaction (PCR) with a primer to a 340bp DNA fragment of BoNT/C (Fach *et al.* 1996). Examination of the spleen or gut wall of EGS cases using this technique may reveal the presence of *C. botulinum* type C.

Classic botulism is usually due to the ingestion of toxin in food and is a particular problem in ranch mink and wildfowl. A vaccine against *C. botulinum* type C toxin is

currently marketed as Botumink (United Vaccines Inc., Madison, Wisconsin, USA); this vaccine has also been used in exotic animals (even sea turtles!) and reportedly in horses (R. Brady, United Vaccines, personal communication), although not under scientifically controlled conditions.

Unfortunately, experience with cell lines has been disappointing (John *et al.* 1997, 2000) and the development of a small animal model, such as the hare, should also be considered; perhaps dysautonomia can be induced in hares by feeding *Fusarium graminearum* and *C. botulinum* type C spores.

So what line of enquiry would Hercule Poirot now embark on? Just as Tocher did 75 years ago, Hunter *et al.* (1999) postulate that grass sickness is a toxicoinfection whereby *C. botulinum* grows in the horse's gut with toxin production *in vivo* which is then absorbed. If Tocher's results on inoculation with the botulinus toxin/anti-toxin mixture are to be believed, then a randomised control trial using 2 doses of vaccine (Botumink?) should be undertaken, preferably in a 'high risk' area in Scotland. Unfortunately, regulatory obstacles for medicinal products and the licensing of investigations, nowadays in place, undoubtedly make a speedy approach of this nature much less practical than in Tocher's era. Nonetheless, reproducing - and improving - Tocher's results is an obvious research target.

One final observation: If Dr Tocher's field work and theories are ultimately vindicated, it will mean that thousands of our equine companions could have been saved from the ravages of grass sickness; it will not be the first time, nor the last, that one man's blind arrogance and stubborn pride put a halt to promising research.

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