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Designing a field trial of an equine grass sickness vaccine: a questionnaire-based feasibility study

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Highlights

- First report of a feasibility study to inform RCT design in veterinary medicine
- 73% of practices had attended ≥1 equine grass sickness (EGS) case in past 2 years
- Higher proportion of EGS-affected premises with recurrent cases in Scotland
- 93% of practices would be willing to participate in a field vaccine trial for EGS
- Low EGS incidence, client factors and paperwork cited as barriers to participation

Abstract

Without an experimental model of Equine Grass Sickness (EGS), a randomised controlled field trial (RCT) represents the only method of evaluating the efficacy of *Clostridium botulinum* type C vaccination in preventing naturally occurring disease. Clinical trial feasibility is an important aspect of preliminary work undertaken prior to initiating RCTs, estimating parameters that are important for study design. This cross-sectional study aimed to assess the feasibility of conducting a nationwide RCT of a candidate vaccine for EGS based on responses from a sample of British equine veterinary practices (n = 119/284).

Seventy-three percent of practices had attended ≥ 1 EGS case within the preceding two years (median four cases), and 51.3% regularly attended recurrently affected premises. Veterinary surgeons had greater confidence diagnosing acute/subacute EGS based solely on history and clinical signs

compared to chronic EGS. Ninety-one percent of respondents (*n* = 103/113) considered the proposed RCT to be important/very important to equine veterinary research. Ninety-one percent of respondents (*n* = 102/112) indicated preparedness to assist in owner recruitment and 92.9% (*n* = 104/112) indicated willingness to participate in a RCT. The most frequent reasons for practices declining to participate were low incidence of EGS (*n* = 4), did not believe clients would wish to participate (*n* = 3) and amount of paperwork/data collection involved (*n* = 2). There was considerable support amongst participating veterinary practices for a RCT evaluating the efficacy of *Clostridium botulinum* vaccination for the prevention of EGS in Britain. Substantial proportions of participating practices would be prepared to participate in the RCT and regularly attended EGS-affected premises that would meet trial inclusion criteria.

Keywords: Clinical trial; Equine grass sickness; Randomised controlled field trial (RCT); Vaccine.

Introduction

Equine grass sickness (EGS) is a predominantly fatal neurodegenerative disease affecting grazing equids, first described in eastern Scotland in the early 1900s (Tocher et al., 1923). Britain continues to have the highest incidence of EGS worldwide (Wylie and Proudman, 2009), with reported incidence rates of 2.1-2.3 cases per 100 horse-years at risk on EGS-affected premises (Newton et al., 2004; Ireland et al., 2011) and an estimated prevalence of 3.2% in areas of Scotland (Doxey et al., 1991a).

It is hypothesised that EGS represents a toxico-infectious form of botulism, with a combination of risk factors resulting in intestinal overgrowth of and neurotoxin production from *Clostridium botulinum* (*C. botulinum*) type C (Newton et al., 2010). Randomised placebo-controlled vaccine field trials conducted in 1922-1923, using an antitoxin-neutralised *C. botulinum* toxin, demonstrated a marked reduction in EGS incidence in vaccinated animals (Tocher, 1924). Lower serum antibody titres to *C. botulinum* type C surface antigens and *C. botulinum* C1 neurotoxin (BoNT/C) were identified in EGS cases compared to controls (Hunter et al., 1999) and a subsequent

case-control study reported that increasing antibody titres to *C. botulinum* type C and BoNT/C toxoid were significantly associated with decreased risk of EGS (McCarthy et al., 2004). Additionally, horses previously in contact with an EGS case were reported to be at reduced risk, potentially indicating that non-fatal exposure to the causative agent may induce some degree of resistance (Wood et al., 1998). Currently, there is no model to reproduce EGS experimentally, precluding the use of experimental challenge studies and therefore a field trial represents the only available method to test the hypothesis that *C. botulinum* type C toxico-infection causes EGS and of evaluating the effect of vaccination in the prevention of naturally occurring disease (Hedderson and Newton, 2004).

The randomised controlled trial (RCT) is considered as the best instrument to evaluate the effectiveness of medical interventions (Oude Rengerink et al., 2010). Clinical trial feasibility is a process of evaluating the possibility of conducting a particular trial in a specific geographical region (Rajadhyaksha, 2010), and is an important first step in initiating a RCT. In human clinical trials, investigator/site selection questionnaires and feasibility checklists are frequently employed to identify potential trial sites and participants. Feasibility studies are considered to be particularly important for RCTs investigating interventions for rare diseases (Hickey et al., 2010).

In order to inform the design of a nationwide randomised, placebo-controlled field trial of a candidate vaccine against EGS in Britain, this cross-sectional feasibility study aimed to identify practices attending premises with high EGS incidence rates and to explore attitudes of veterinary surgeons towards the proposed RCT.

Materials and methods

Selection of study sample

Non-probability sampling was used, with all veterinary practices (n = 200) registered with a nationwide EGS surveillance scheme covering England, Scotland and Wales (Wylie et al., 2011) being invited to participate. Additionally, from the database of referring veterinary practices held by the Diagnostic Laboratory Services at the Animal Health Trust, a further 84 practices (located in

England, Scotland and Wales) with equine clients were identified and the principal partners were invited to participate.

Questionnaire design

The self-administered postal questionnaire was designed using an automated data capture system (Autonomy, TeleForm version 10.2) (Supplementary material 1). The questionnaire contained a study synopsis pertaining to the proposed protocol for a nationwide RCT of a candidate vaccine for EGS. The questionnaire was pretested amongst a group of veterinary surgeons, who were not enrolled on the study, and revised in accordance with their comments. The questionnaire was accompanied by a reply-paid envelope and a hand-signed covering letter that assured confidentiality and provided the principal investigator's name, address, telephone number and email address. To maximise response rates, reminder postcards were sent to non-respondents 8 weeks after the initial mailing, followed by a second questionnaire mailing 6 weeks after the reminder postcards to remaining non-respondents.

Data analysis

Questionnaire data were scanned and verified using TeleForm then exported to Microsoft Excel. Statistical analyses were performed using commercial software (SPSS version 21). Data are described as medians with interquartile ranges (IQR) for continuous data and as proportions with 95% confidence intervals (CI) for categorical data. Pearson Chi-squared or Fisher's exact tests were used to assess associations between categorical variables. Kruskal-Wallis or Mann-Whitney *U* tests were used to test the statistical significance of differences in median values of continuous variables between categories of categorical variables. Critical probability was set at 0.05.

Results

Description of responses

Of the 284 questionnaires mailed, 119 useable responses (41.9%) were returned, with a further three non-useable responses received from practices declining to participate due to limited numbers of EGS cases seen by the practice. Detailed descriptions of responses and characteristics of

responding practices are available as supplementary information (items 2 and 3). Comparison of respondents with non-respondents found no association between response rate and country (England, Scotland or Wales) ($P = 0.40$), type of practice ($P = 0.13$) or registration with the EGS surveillance scheme ($P = 0.10$).

Of the 119 responding practices, 2.5% ($n = 3$; 0-5.3%) no longer undertook equine work, and these responses were excluded from further data analysis. Thirty-eight percent ($n = 44/116$; 29.1-46.8%) of practices were equine-only; 49.1% ($n = 57$; 40.0-58.2%) were mixed practices with < 50% equine work and 12.9% ($n = 15$; 6.8-19.0%) were mixed practices with ≥ 50% equine work. The majority were first opinion practices (69.8%; $n = 81$; 61.5-78.2%). The number of registered horses/ponies differed with proportion of equine work: equine-only practices had a median of 5,250 horses/ponies (IQR 2,075-10,000), mixed practices with ≥ 50% equine work had a median of 4,812 horses/ponies (IQR 1,500-13,684) and mixed practices with < 50% equine work had a median of 1,000 horses/ponies (IQR 358-2,000) ($P < 0.001$).

Veterinary surgeon experience of EGS

The majority of respondents reported that their practice had attended ≥ 1 EGS case within the preceding two years (73.0%; $n = 84/115$; 64.9-81.1%; Figure 1), with a median of four cases (IQR 2-7; range 1-20). A greater proportion of equine-only practices (90.7%; $n = 39/43$; 82.0-99.4%) reported attending ≥ 1 EGS case within the preceding two years compared to mixed practices (61.6%; $n = 45/73$; 50.5-72.8%) ($P = 0.002$). Using the estimated total number of registered horses/ponies, the median EGS period prevalence for the preceding two years was 0.08% (IQR 0.005-0.25%; range 0-3.0%). The period prevalence in Scotland was higher (median 0.5%; IQR 0.25-1.0%) compared to England (median 0.05%; IQR 0.005-0.17%) or Wales (median 0.03%; IQR 0.005-0.07%) ($P = 0.005$). Excluding practices reporting no EGS cases in the previous two years, the median period prevalence was 0.12% (IQR 0.02-0.3%; range 0.009-3.0%).

The majority of respondents indicated they could readily identify premises attended by their practice that had been affected by EGS within the preceding two years (68.8%; *n* = 77/112; 60.2-77.3%), with a greater proportion of equine-only practices (81.0%; *n* = 34/42; 69.1-92.8%) able to identify EGS-affected premises compared to mixed practices (61.4%; *n* = 43/70; 50.0-72.8%) (*P* = 0.03). Fifty-one percent of respondents (*n* = 56/109; 42.0-60.8%) indicated their practice regularly attended premises recurrently affected by EGS. There was an association between country and recurrent premises (*P* = 0.001), with 92.3% of practices in Scotland (*n* = 12/13; 77.8-100%) able to identify recurrent premises, compared to 47.8% of practices in England (*n* = 44/92; 37.6-58.0%) and none of the practices in Wales (*n* = 0/4; 0-0.49%).

The majority of respondents reported that their practice provided recommendations for management on EGS-affected premises. The most frequently recommended preventive management strategies were minimising pasture disturbance, removal of horses/ponies from affected fields for a specified period of time and prioritising preventive measures at high risk times of year and/or for high risk groups of horses/ponies (Table 1).

Respondents indicated that veterinary surgeons within their practice were more confident diagnosing the acute or subacute clinical subtypes of EGS based solely on history and clinical signs compared to cases of chronic EGS (Table 2). For diagnosis of acute/subacute EGS, a greater proportion of respondents from equine-only practices (81.4%; *n* = 35/43; 69.8-93.0%) indicated that veterinary surgeons within their practice were confident/very confident based solely on history and clinical signs compared to respondents from mixed practices (63.0%; *n* = 46/73; 51.9-74.1%) (*P* = 0.04). Similarly, for diagnosis of chronic EGS, a greater proportion of respondents from equine-only practices (69.8%; *n* = 30/43; 56.0-83.5%) indicated that veterinary surgeons within their practice were confident/very confident based solely on history and clinical signs compared to respondents from mixed practices (45.1%; *n* = 32/71; 33.5-56.6%) (*P* = 0.01). For diagnosis of acute/subacute EGS, the median number of EGS cases attended within the preceding two years was greater where veterinary surgeons were reported to be confident/very confident compared to those reported to be not/somewhat

confident (median 4 cases, IQ 2 – 7 cases and median 2, IQ 0 – 3 cases, respectively) ($P < 0.001$). The median number of EGS cases attended within the preceding two years was also greater where veterinary surgeons were reported to be confident/very confident in the clinical diagnosis of chronic EGS compared to those reported to be not/somewhat confident (median 5 cases, IQ 2 – 9 cases and median 2, IQ 0 – 4 cases, respectively) ($P < 0.001$). The most frequently reported *ante-mortem* ancillary diagnostic tests used in the investigation of suspected cases of EGS were phenylephrine eye drops and routine haematology and biochemistry (Table 2).

Potential participation in EGS vaccine RCT

When asked about the feasibility of undertaking certain aspects of clinical assessments, treatment administration and data collection, the majority of respondents indicated that the proposed RCT protocol would be feasible (Table 3).

Overall, 99.1% of respondents (n = 111/112; 97.4-100%) indicated willingness to participate in the RCT if a client registered with their practice wished to enrol, and 72.3% of respondents (n = 81/112; 64.0-80.6%) indicated that they would recommend participation in the RCT to all clients keeping horses/ponies on EGS-affected premises, with a further 26.8% (n = 30/112; 18.6-35.0%) indicating that they would recommend participation to selected equine clients.

The majority of respondents (85.8%; n = 91/106; 79.2-92.5%) indicated that if they owned a horse/pony they would be willing to enrol them in the RCT. Similarly, 85.5% of respondents (n = 94/110; 78.9-92.0%) indicated that they would be prepared to enrol a horse/pony owned by a family member or close friend in the RCT. Respondents believed the proposed RCT was of greatest importance for equine veterinary research, with a lower proportion of respondents considering the RCT was important to their practice equine population (Figure 2). Should a vaccine demonstrated to be effective in the prevention of EGS be available, 48.6% of respondents (n = 54/111; 39.3-57.9%) would recommend its use to all equine clients registered with their practice. A further 37.8% (n = 42/111; 28.8-46.9%) would recommend vaccination to all clients keeping horses/ponies on EGS-

affected premises and 11.7% (*n* = 13/111; 5.7-17.7%) would recommend vaccination to selected clients.

Ninety-one percent of respondents (*n* = 102/112; 85.8-96.4%) indicated preparedness to assist in recruitment of owners for the RCT. Overall, 92.9% of respondents (*n* = 104/112; 88.1-97.6%) indicated willingness to participate in the RCT. Reasons given by the eight negative respondents were: the RCT was not relevant to practice caseload/low EGS incidence (*n* = 4); they did not believe clients would wish to participate (*n* = 3); they considered that there would be too much paperwork involved (*n* = 2); the RCT was too great a time commitment (*n* = 1); they were not interested in the RCT/EGS (*n* = 1); concerns over causal association between *C. botulinum* type C and EGS and limited available safety data (*n* = 1); and forthcoming personnel changes at the practice (*n* = 1).

Discussion

Clinical trial feasibility studies are not widely used in veterinary medicine, yet a site feasibility survey represents a small expenditure, in terms of both time and financial cost, and can provide invaluable information to inform RCT study design. The key findings from this feasibility study are that the majority of participating veterinary practices could readily identify EGS-affected premises and would be prepared to consider entering animals under their care into an RCT investigating the efficacy of *C. botulinum* type C vaccination in the prevention of naturally occurring EGS. This study also provided important information about reasons why veterinary surgeons may not wish to enter this trial. To the authors' knowledge, this is the first report of using a site feasibility study to inform the design of a RCT in veterinary medicine.

Non-probability sampling was used to identify the accessible population of veterinary practices invited to participate in this study, thereby introducing selection bias. However, purposive sampling of all veterinary practices registered with the EGS surveillance scheme (Wylie et al., 2011) facilitated assessment of the usefulness of the scheme for recruitment of horses to the proposed EGS RCT. The useable response rate to the postal questionnaires of 41.9% was disappointing and may

have introduced further selection bias; however it is comparable to response rates achieved in other questionnaire surveys of equine veterinary surgeons (Savage et al., 1998; Price et al., 2002; Hewson et al., 2007; Mair and White, 2008).

With the aim of maximising response rate and minimising non-response bias, many elements of the tailored design method (Dillman, 2007) were utilised in the administration of this survey. These included the use of personalised cover letters, sending questionnaires by first class post and providing non-respondents with a second copy of the questionnaire, which were all reported in a systematic review as methods that significantly increase response rates to postal questionnaires (Edwards et al., 2002). The risk of errors introduced by responder bias is a well-recognised limitation of all questionnaire-based research and in this study respondents are likely to be individuals with a particular interest in EGS, and not, therefore, a representative sample of the equine veterinary profession in the UK. Although not statistically significant, comparison of respondents with non-respondents identified that a greater proportion of practices registered with the EGS surveillance scheme responded than other practices invited to participate in the study. However, it is unlikely that practices rarely attending cases of EGS would elect to register with the surveillance scheme and practices in regions with lower EGS incidence are likely to be under-represented in this study. Although this degree of response bias precludes direct extrapolation of this study's findings to all veterinary practices undertaking equine work in Britain, it does support use of the surveillance scheme in recruiting practices as site investigators for the proposed EGS RCT.

Both equine-only and mixed practices were represented in the study population, with the majority of respondents working in solely first opinion practices. As might be expected, equine-only practices had greater numbers of registered horses, and a larger proportion had attended EGS cases within the study period. *Ante-mortem* diagnosis of EGS is often presumptive, based on a combination of historical epidemiological information and clinical signs. However clinical signs exhibited are often diverse, varying with disease severity, and no clinical sign is pathognomonic for all forms of the disease (Doxey et al., 1991b). In addition, many of the clinical signs observed may also occur in a

substantial proportion of colic cases (Doxey et al., 1991b), and it may be difficult to differentiate acute/subacute EGS from other causes of colic particularly in areas where the disease is less prevalent (Milne, 1996). In this study, respondents indicated a greater degree of confidence making a clinical diagnosis of acute/subacute EGS compared to cases of chronic EGS. Veterinary surgeons from equine-only practices were more confident in the clinical diagnosis of EGS compared to those from mixed practices, which may reflect the increased likelihood of these respondents having recent experience of the disease. Furthermore, veterinary surgeons that were confident in diagnosing EGS based solely on history and clinical signs had attended a greater number of EGS cases within the preceding two years.

Prevalence of EGS was greatest in Scotland, consistent with historical reports (Guthrie, 1940; Gilmour and Jolly, 1974), and in keeping with more recent data, a significantly greater proportion of Scottish practices regularly attended EGS-affected premises with a history of disease recurrence (Wylie et al., 2011). Given the small proportion of respondents indicating that suspected cases of EGS attended by their practice were definitively diagnosed via histopathology, misclassification bias may have resulted in overestimation of EGS prevalence in this study. Additionally, diagnostic suspicion bias, where exposure is taken as a diagnostic criterion, may influence the EGS prevalence reported in the current study (Delgado-Rodríguez and Llorca, 2004; Sackett 1979). For example, veterinary surgeons' knowledge of a horse's prior exposure to EGS risk factors, particularly on recurrently affected premises, may influence their subsequent diagnostic process where EGS is suspected. However, where veterinary surgeons have experience of EGS, diagnostic accuracy based on signalment, historical and clinical findings, is considered to be high (Pirie 2006), and accuracy of clinical diagnosis in cases of chronic EGS has been reported as 100% (Doxey et al., 1998).

Numerous epidemiological studies have identified an array of risk factors for EGS (recently reviewed by Pirie et al. (2014)), and in the absence of any available preventive healthcare measure current recommendations focus on implementation of management strategies designed to minimise exposure to risk factors. The majority of respondents in this study provided management advice for

EGS-affected premises, predominantly pertaining to pasture management, reducing access to the EGS-affected paddock and prioritising preventive management strategies for high risk animals, and particularly where respondents had attended EGS cases within the preceding 2 years. Premises where pasture had been disturbed, for example through construction work or moles, within the previous 12 months had higher odd of an EGS case occurring compared to pastures that had not been disturbed (odd ratio 3.4) (McCarthy et al., 2004b), and minimising pasture disturbance and soil exposure was the most frequent recommendation by respondents in this study. Over 30% of respondents advised some degree of restricted grazing of EGS-affected paddocks, consistent with several studies reporting increased risk of EGS occurrence with access to grazing, particularly on pastures with a previous history of EGS (Gilmour and Jolly, 1974; Doxey et al., 1991; Wood et al., 1998; McCarthy et al., 2004a). Previous studies have reported increased risk of EGS in young adults (Gilmour and Jolly, 1974; Doxey et al., 1991; Wood et al., 1998; McCarthy et al., 2004a; Newton et al.; 2004), and in animals that have recently moved to new premises or pasture (Gilmour and Jolly, 1974; Doxey et al., 1991; Wood et al., 1998; McCarthy et al., 2004a), and 42% of respondents advised prioritising preventive management strategies for animals within these higher risk groups. It is likely that a substantial proportion of EGS-affected premises will implement preventive management strategies in order to try to reduce the risk of recurrence. his needs to be taken into consideration as a potential bias when designing protocols for any intervention for EGS, including a vaccine RCT. An appropriately conducted RCT, with random treatment group allocation performed at premises level, would facilitate controlling for these management-level risk factors.

Poor investigator compliance with trial protocols can have important effects on the overall result (Prescott et al., 1999). Poor design of data collection methods, excessive data collection and follow-up have been cited by clinicians as impediments to patient recruitment in human trials (Benson et al., 1991; Coombs et al., 1993). In the current study, of the small number of respondents not willing to participate in the proposed EGS RCT, 25% considered that there would be too much paperwork involved. The majority of respondents indicated that data collection, clinical assessments and treatment administration aspects of the proposed RCT protocol would be feasible for their practice to

undertake (Table 3), implying that attaining good compliance with the trial protocol would be achievable. A substantial proportion of respondents indicated that provision of clerical support or additional remuneration would be required for data collection, and that availability of veterinary support for the treatment administration phase would be desirable. Ensuring these factors are considered in both the design and financial requirements for the proposed RCT will help to maximise veterinary investigators' compliance with the trial protocol.

In a survey of healthcare professionals, 17% indicated that scientifically uninteresting trials were an impediment to recruitment (Foley and Moertel, 1991). Questions addressed by RCTs should be interesting and relevant to practice (Fletcher et al., 2012) and of sufficient importance to clinicians for them to be willing to take part and comply with protocol requirements (Prescott et al., 1999). While only 49% of respondents in the current study considered a nationwide RCT of a candidate vaccine against EGS would be important to their own practice, 91% considered that this RCT would be important to equine veterinary research.

The majority of respondents indicated willingness to assist in recruitment of horse owners for the proposed RCT, and a large proportion indicated that they would recommend participation to owners registered with their practice should one of their clients enrol in the trial. The reasons given by those not wishing to aid in the recruitment phase were consistent with factors frequently reported to act as barriers to the recruitment activity of clinicians in human clinical trials (Prescott et al., 1999; Ross et al., 1999). In order to assess the overall acceptability of the proposed RCT, respondents were asked whether or not they would enrol their own animal, or a horse/pony owned by a family member or close friend, with the majority responding positively to both scenarios.

Ninety-three percent of respondents indicated willingness to participate in the RCT, with a greater proportion indicating that they would take part should a client wish to enrol. Most respondents to the current study indicated that they would recommend the use of vaccination in the prevention of EGS, should an effective vaccine be available. As with barriers to recruitment, reasons given by

respondents for not wishing to participate in the proposed RCT were broadly similar to factors affecting clinician decisions regarding taking part in clinical trials (Prescott et al., 1999; Ross et al., 1999). Clinician concerns about adverse effects of treatment or the burden to patients were cited as important factors in deciding whether or not to take part in cancer clinical trials (Foley and Moertel, 1991). While these factors were considered important barriers by a very low number of respondents in this study, ensuring safety data are available and addressing protocol-related barriers to owner participation should be incorporated in the design of the proposed RCT.

Conclusions

The results of this study indicate that undertaking a RCT evaluating the efficacy of *C. botulinum* vaccination for the prevention of EGS in Britain would be feasible, with considerable support for such a trial demonstrated amongst participating veterinary surgeons. Results provided an estimate of the proportions of practices attending EGS-affected premises that would meet inclusion criteria and would be prepared to participate in the proposed RCT, both of which were high despite a low overall estimated prevalence of EGS. The study also provided information regarding aspects of trial design that might make it more acceptable.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. With the exception of institutional ethical approval from the Animal Health Trust Clinical Research Ethics Committee (AHT09-2012), funding sources had no involvement in study design, conduct of the study, analysis and interpretation of data, preparation of the manuscript or in the decision to submit the article for publication.

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Appendix: Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:

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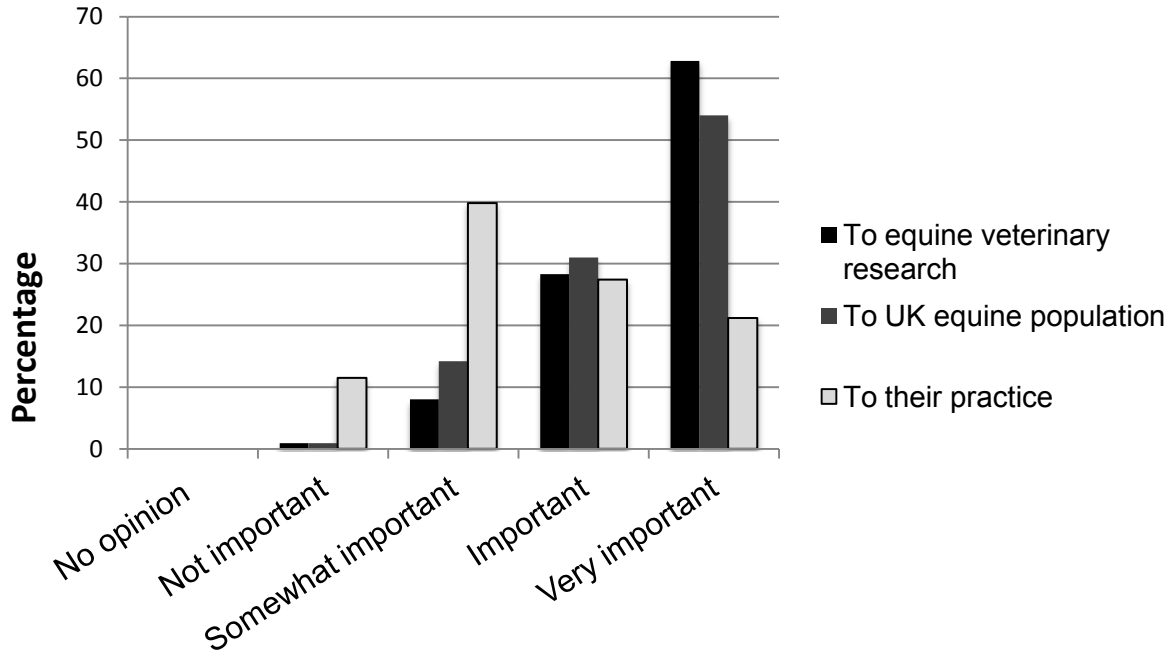
Figure legends

Figure 1: Map of the geographical distribution of veterinary practices, attending no or ≥1 EGS cases within the preceding two years, participating a survey of veterinary surgeons in Britain (*n*=116).

Figure 2: Veterinary surgeons' opinions regarding the potential importance of a proposed RCT of a vaccine for the prevention of EGS reported in a survey of veterinary surgeons in Britain (*n*=113).



518 **Table 1:** Preventive management measures currently recommended for EGS-affected premises in a
519 survey of veterinary surgeons in Britain (*n*=116).

Preventive management strategies recommended for EGS-affected premises	**Frequency** (all respondents *n*=113)	**Percent (95% CI)**	**Frequency** (only respondents seeing EGS in last 2 years n=84)	**Percent (95% CI)**
None	18	15.9 (9.2-22.7)	7	8.4 (2.4-14.2)
Remove horses permanently from affected paddock/field	21	18.6 (11.4-25.8)	13	15.7 (7.7-23.2)
Remove horses from affected paddock/field for specified time period	59	44.2 (43.0-61.4)	44	53.0 (41.7-63.1)
Reduce time spent grazing affected paddock/field	34	30.1 (21.6-38.5)	30	36.1 (25.5-46.0)
Avoid sudden dietary changes	41	36.3 (27.4-45.1)	34	41.0 (30.0-51.0)
Provide supplementary forage	42	37.2 (28.2-46.1)	37	44.6 (33.4-54.7)
Avoid overuse of ivermectin anthelmintics	17	15.0 (8.5-21.6)	16	19.3 (10.6-27.4)
Co-graze with ruminants	20	17.7 (10.7-24.7)	19	22.9 (13.7-31.6)
Avoid stressful incidents	29	25.7 (17.6-33.7)	22	26.5 (16.8-35.6)
Minimise soil exposure and pasture/soil disturbance	69	61.1 (52.1-70.1)	56	67.5 (56.6-76.7)
Hand removal of faeces (not mechanical)	38	33.6 (24.9-42.3)	29	34.9 (24.4-44.7)
Prioritise preventive measures at high risk times of year	45	39.8 (30.8-48.8)	39	47.0 (35.8-57.1)
Prioritise preventive measures for high risk animals (e.g. young adults, new arrivals)	47	41.6 (32.5-50.7)	40	48.2 (36.9-58.3)
Other preventive measure*	6	5.3 (1.2-9.4)	6	7.2 (1.6-12.6)

520 *Other preventive measures included calling EGS Fund for latest advice; minimise time spent on wet/flooded
521 areas; monitor stock density vs sward height; move to new premises; mechanical faecal removal employed and
522 providing supplementary selenium and limestone flour.
523

524 **Table 2:** Level of confidence in diagnosis of EGS based on case history and clinical signs alone and
525 ancillary diagnostic test utilised in the investigation of suspected EGS cases reported in a survey of
526 veterinary surgeons in Britain (*n*=116).

Level of confidence in diagnosis based on history and clinical signs alone	**Acute/ Subacute (*n*=116) Frequency (%; 95% CI)**	**Chronic (*n*=114) Frequency (%; 95% CI)**
Not confident	4 (3.4; 0.1-6.8)	9 (7.9; 2.9-12.8)
Somewhat confident	31 (26.7; 18.7-34.8)	43 (37.7; 28.8-46.6)
Confident	53 (45.7; 36.6-54.8)	44 (38.6; 29.7-47.5)
Very confident	28 (24.1; 16.4-31.9)	18 (15.8; 9.1-22.5)
Ancillary/diagnostic test used in the investigation of suspected EGS cases	**Acute/ Subacute (n=87) Frequency (%)**	**Chronic (n=84) Frequency (%)**
None	2 (2.3; 0-5.4)	4 (4.6; 0.2-9.3)
Phenylephrine eye drops	42 (48.3; 37.8-58.8)	42 (50.0; 39.3-60.7)
Routine haematology/biochemistry	24 (27.6; 18.2-37.0)	31 (35.6; 26.6-47.2)
Nasogastric intubation	23 (26.4; 17.2-35.7)	10 (11.5; 5.0-18.8)
Exploratory laparotomy +/- ileal biopsy	20 (23.0; 14.1-31.8)	21 (24.1; 15.7-34.3)
Rectal examination	17 (19.5; 11.2-27.9)	10 (11.5; 5.0-18.8)
Abdominocentesis	17 (19.5; 11.2-27.9)	16 (18.4; 10.6-27.4)
Post mortem examination	14 (16.1; 8.4-23.8)	5 (5.7; 0.9-11.0)
Abdominal ultrasonography	4 (4.6; 0.2-9.0)	6 (6.9; 1.6-12.6)
Endoscopy	4 (4.6; 0.2-9.0)	4 (4.6; 0.2-9.3)
Rectal biopsy	2 (2.3; 0-5.4)	5 (5.7; 0.9-11.0)
Faecal worm egg count	2 (2.3; 0-5.4)	2 (2.3; 0-5.6)
Other ancillary/diagnostic tests*	3 (3.4; 0-7.3)	11 (12.6; 5.9-20.3)

527 *Other ancillary/diagnostic tests for acute/subacute EGS cases included barium oesophogram, faecal analysis
528 and lack of response to treatment (all *n*=1); other ancillary/diagnostic tests for chronic EGS cases included
529 gastroscopy/gastroduodenoscopy (*n*=2), oral glucose absorption test (*n*=4), faecal analysis (*n*=2), barium
530 oesophogram (*n*=1), full dental examination (*n*=1) and weight loss investigation (*n*=1).
531

532 **Table 3:** Veterinary surgeons' opinions regarding aspects of data collection, clinical assessment and
533 treatment administration for a proposed RCT of a vaccine for the prevention of EGS reported in a
534 survey of veterinary surgeons in Britain (*n*=116).

Veterinary surgeon participation in proposed EGS vaccine field trial (RCT)	**Frequency (%; 95% CI)**		
	Yes	**Yes, with additional support and/or fees provided**	**No**
Would it be feasible for veterinary surgeons at your practice to complete standardised recording forms for each clinical examination during the RCT? (*n*=114)	67 (58.8; 49.7-67.8)	45 (39.5; 30.5-48.4)	2 (1.8; 0-4.2)
Would it be feasible for veterinary surgeons at your practice to complete standardised recording forms for any suspected adverse event during the RCT? (*n*=114)	54 (47.4; 38.2-56.5)	58 (50.9; 41.7-60.1)	2 (1.8; 0-4.2)
Would it be feasible for veterinary surgeons at your practice to inform RCT staff immediately regarding any suspected EGS cases attended? (*n*=114)	114 (100.0; 96.8-100.0)	N/A	0
Would it be feasible for veterinary surgeons at your practice to inform RCT staff immediately regarding any cases of mortality occurring in horses/ponies enrolled in the RCT? (*n*=113)	113 (100.0; 96.8-100.0)	N/A	0
Would your practice be willing to help RCT staff facilitate the collection and transportation of fatal cases of suspected EGS for *post mortem* examination? (*n*=113)	108 (95.6; 91.8-99.4)	N/A	5 (4.4; 0.6-8.2)
	Yes	**Yes, only if accompanied by vet from practice**	**No**
For suspected EGS cases, would your practice allow additional clinical examinations undertaken by RCT staff? (*n*=113)	92 (81.4; 74.2-88.6)	21 (18.6; 11.4-25.8)	0
	All treatments administered by practice vets	**All treatments administered by practice vets with support from locums as required**	**All treatments administered by dedicated, specifically trained RCT locum vet**
Which option would you prefer for administration of RCT treatments for horses/ponies under the care of your practice? (*n*=110)	51 (46.4; 37.0-55.7)	57 (51.8; 42.5-61.2)	2 (1.8; 0-4.3)

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